PI: **DORE-DUFFY, PAULA**	Title: Induction of physiological adaptive angiogenesis is protective in a model of trau	
Received: 06/05/2009	FOA: PA08-015	Council: 01/2010
Competition ID: ADOBE-FORMS-A	FOA Title: ANGIOGENESIS IN THE NERVOUS SYSTEM IN HEALTH AND DISEASE (R01)	
1 R01 NS069937-01	Dual:	Accession Number: 3199199
IPF: 9110501	Organization: WAYNE STATE UNIVERSITY	
Former Number:	Department: Neurology	
IRG/SRG: ANIE	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 372,379 Year 2: 319,536 Year 3: 329,122 Year 4: 338,994 Year 5: 349,164	Animals: Y Humans: N Clinical Trial: N Current HS Code: 10 HESC: N	New Investigator: N Early Stage Investigator: N

Senior/Key Personnel:	*Organization:*	*Role Category:*
Paula Dore-Duffy Ph.D.	Wayne State University	PD/PI
Jose Rafols PhD	Wayne State University	Other (Specify)-Co-Principle Investigator
Christian Kreipke PhD	Wayne State University	Other (Specify)-Co-Principle Investigator
Alexander Gow PhD	Wayne State University	Other (Specify)-Co-Principle Investigator
Joseph LaManna PhD	Case Western Reserve University	Consultant

Appendices

Angiodynamics review 2007, Cns pcs stem cell 2006, Vegf isoforms, Imp rough draft june09, Shen et al 2007, Pc review

Additions for Review

Supplemental Material	Supplemental	08/31/2009

APPLICATION FOR FEDERAL ASSISTANCE

SF 424 (R&R)

2. DATE SUBMITTED	Applicant Identifier
3. DATE RECEIVED BY STATE	**State Application Identifier**

1. * TYPE OF SUBMISSION

☐ Pre-application ☒ Application ☐ Changed/Corrected Application

4. Federal Identifier

5. APPLICANT INFORMATION

*** Organizational DUNS:** 001962224

* Legal Name: Wayne State University

Department: Sponsored Programs Admin Division:

* Street1: 5057 Woodward

Street2: Suite 6402

* City: Detroit County: Wayne

* State: MI: Michigan Province:

* Country: USA: UNITED STATES * ZIP / Postal Code: 48202

Person to be contacted on matters involving this application

Prefix: Mrs. * First Name: Gail Middle Name:

* Last Name: Ryan Suffix:

* Phone Number: 313-577-1445 Fax Number: 313-577-1348

Email: orspsmail@wayne.edu

6. * EMPLOYER IDENTIFICATION *(EIN) or (TIN):* 386028429

7. * TYPE OF APPLICANT: H: Public/State Controlled Institution of Higher Education

Other (Specify):

Small Business Organization Type ☐ Women Owned ☐ Socially and Economically Disadvantaged

8. * TYPE OF APPLICATION:

☒ New ☐ Resubmission

☐ Renewal ☐ Continuation ☐ Revision

If Revision, mark appropriate box(es).

☐ A. Increase Award ☐ B. Decrease Award ☐ C. Increase Duration ☐ D. Decrease Duration

☐ E. Other (specify):

* Is this application being submitted to other agencies? Yes ☐ No ☒ What other Agencies?

9. * NAME OF FEDERAL AGENCY:

National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER:

TITLE:

11. * DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:

Induction of physiological adaptive angiogenesis is protective in a model of traumatic brain injury

12. * AREAS AFFECTED BY PROJECT *(cities, counties, states, etc.)*

Detroit, Wayne County, Michigan

13. PROPOSED PROJECT:

* Start Date: 04/01/2010 * Ending Date: 03/31/2015

14. CONGRESSIONAL DISTRICTS OF:

a. * Applicant: MI-013 b. * Project: MI-013

15. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: Dr. * First Name: Paula Middle Name:

* Last Name: Dore-Duffy Suffix: Ph.D.

Position/Title: Professor

* Organization Name: Wayne State University

Department: Neurology Division:

* Street1: 421 East Canfield

Street2: 3126 Elliman Building

* City: Detroit County: Wayne

* State: MI: Michigan Province:

* Country: USA: UNITED STATES * ZIP / Postal Code: 48201

* Phone Number: 313-577-0354 Fax Number: 313-577-7552

* Email: pdduffy@med.wayne.edu

OMB Number: 4040-0001
Expiration Date: 04/30/2008

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

16. ESTIMATED PROJECT FUNDING

a. * Total Estimated Project Funding	1,709,195.00
b. * Total Federal & Non-Federal Funds	1,709,195.00
c. * Estimated Program Income	0.00

17. * IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?

a. YES ☐ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ☒ PROGRAM IS NOT COVERED BY E.O. 12372; OR

☐ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

18. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

☒ * I agree

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

19. Authorized Representative

Prefix:	Miss
* First Name:	Tamara
Middle Name:	
* Last Name:	Heaton
Suffix:	
* Position/Title:	Grants Officer III
* Organization:	Wayne State University
Department:	Sponsored Programs Admin
Division:	
* Street1:	4201 St. Antoine
Street2:	9D UHC
* City:	Detroit
County:	Wayne
* State:	MI: Michigan
Province:	
* Country:	USA: UNITED STATES
* ZIP / Postal Code:	48201-1998
* Phone Number:	313-577-9342
Fax Number:	
* Email:	theatonb@med.wayne.edu

*** Signature of Authorized Representative**: Tamara Heaton-Bauer

*** Date Signed**: 06/05/2009

20. Pre-application Add Attachment | Delete Attachment | View Attachment

21. Attach an additional list of Project Congressional Districts if needed.

Add Attachment | Delete Attachment | View Attachment

OMB Number: 4040-0001
Expiration Date: 04/30/2008

424 R&R and PHS-398 Specific Table Of Contents

RESEARCH & RELATED Project/Performance Site Location(s)

Project/Performance Site Primary Location

Organization Name: Wayne State University
* Street1: 421 East Canfield Avenue
Street2: Elliman Building - Rms 2125,2205,3121,3125
* City: Detroit County: Wayne
* State: MI: Michigan Province:
* Country: USA: UNITED STATES * ZIP / Postal Code: 48201

Project/Performance Site Location 1

Organization Name: Wayne State University
* Street1: 540 East Canfield Avenue
Street2: Scott Hall - Rms 9312,9320
* City: Detroit County: Wayne
* State: MI: Michigan Province:
* Country: USA: UNITED STATES * ZIP / Postal Code: 48201

Additional Location(s) Add Attachment Delete Attachment View Attachment

OMB Number: 4040-0001
Expiration Date: 04/30/2008

Close Form | Print Page | About

RESEARCH & RELATED Other Project Information

1. * Are Human Subjects Involved? ☐ Yes ☒ No

1.a If YES to Human Subjects

Is the IRB review Pending? ☐ Yes ☐ No

IRB Approval Date:

Exemption Number: ☐1 ☐2 ☐3 ☐4 ☐5 ☐6

Human Subject Assurance Number:

2. * Are Vertebrate Animals Used? ☒ Yes ☐ No

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ☒ Yes ☐ No

IACUC Approval Date:

Animal Welfare Assurance Number A3310-01

3. * Is proprietary/privileged information included in the application? ☐ Yes ☒ No

4.a. * Does this project have an actual or potential impact on the environment? ☐ Yes ☒ No

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ☐ Yes ☐ No

4.d. If yes, please explain:

5.a. * Does this project involve activities outside the U.S. or partnership with International Collaborators? ☐ Yes ☒ No

5.b. If yes, identify countries:

5.c. Optional Explanation:

6. * Project Summary/Abstract 1239-Abstract o6 o9 grant.pdf | Add Attachment | Delete Attachment | View Attachment

7. * Project Narrative 1242-Relevance o6o9.pdf | Add Attachment | Delete Attachment | View Attachment

8. Bibliography & References Cited 1241-Bibliography NIH o6o9.pdf | Add Attachment | Delete Attachment | View Attachment

9. Facilities & Other Resources 1240-F & R NIH June 2009.pdf | Add Attachment | Delete Attachment | View Attachment

10. Equipment | Add Attachment | Delete Attachment | View Attachment

11. Other Attachments Add Attachments | Delete Attachments | View Attachments ☐

OMB Number: 4040-0001
Expiration Date: 04/30/2008

An alarming number of civilians as well as the military personnel suffer from the neurological and cognitive consequences of traumatic brain injury (TBI). TBI is now the signature injury seen in troops serving in combat operations in Irag. The U.S. Dept of Health and Human Services reports over half of injured U.S. soldiers are diagnosed with TBI. Direct and indirect costs, including lost productivity total over $60 billion annually and produce devastating affects on the American family dynamics.

While the primary pathophysiological events associated with TBI, diffuse axonal injury (DAI) cognitive damage, cerebral blood flow and neuronal death, have been reported, therapeutic efforts have proved disappointing. This may be due to a lack of fundamental knowledge on how to maintain CNS homeostasis or to buffer the brain from secondary injury following TBI. Maintenance of CNS homeostasis is a coordinated effort of cellular constituents in the brain. These cells make fine tuned regulatory adjustments that include induction of physiological adaptive angiogenesis. Our laboratories have observed and characterized the angiogenic response following TBI, hypoxia (normobaric and hyperbaric), and have reported that adaptive angiogenesis may be deregulated. Induction of therapeutic angiogenesis post-injury promotes HIF-1α and ameliorates cognitive decline in injured animals. Our results suggest that although aspects of angiogenesis are induced following TBI, vascular remodeling is ineffective and the balance between metabolic need, and tissue oxygen and glucose availability is disrupted. **We hypothesize that restoration or normalization of angiodynamics will augment neuro-protection and CNS repair, mitigating the extent of secondary injury and sparing cognitive deficit following TBI. In the present application, we propose that therapeutic angiogenesis (exposure to normobaric hypoxia) stabilizes HIF-1α, promotes physiological angiogenesis and has a profound affect on long-term recovery following TBI. Three aims will be tested: (1.) Characterize angiodynamics, neuronal injury, and cognitive deficits following TBI; (2.) Determine the effect of normobaric hypoxia on angiodynamics and neuropathologic/cognitive outcome following TBI; (3.) Characterize angiodynamics following exposure to chronic mild normobaric to determine the mechanistic underpinnings of neuroprotection and stabilization of angiodynamics produced by normobaric hypoxia.**

Relevance of Proposal to Public Health.

The U.S. Department of Health and Human Services (2005, October) estimates that nearly two-thirds of injured U.S. soldiers sent to Walter Reed Army Medical Center were diagnosed with traumatic brain injury (TBI) and therapeutic efforts have proved disappointing. **We hypothesize that restoration or normalization of dysfunctional angiodynamics will enhance neuroprotection, mitigate the extent of secondary injury, and spare cognitive deficit following TBI. In the present application, we propose that therapeutic intervention using exposure to mild hypoxia is a noninvasive, low-cost approach that will promote adaptive changes in the brain and will have a profound affect on clinical outcome and long-term recovery.**

Facilities and Resources

Laboratory

1) Dore-Duffy's two laboratories are located on the third floor of Elliman and occupy approximately 1500 sq ft. The labs are fully equipped and staffed for cell culture, immunocytochemistry, hypoxia experiments, animal dissection, image analysis, and molecular biological experiments.
2) Dr. Gow's cellular/molecular biology labs occupy 1200 sq ft on the second floor of the Elliman Building. They are fully functional and staffed for DNA cloning, Southern and PCR genotyping, expression analyses in mammalian cells/tissues by RNA, protein, immunochemistry, histochemistry, histology, and radiochemical work.
3) Dr. Rafols' laboratory is located on the ninth floor of Scott Hall and occupies 1280 sq ft. It is fully equipped and staffed for immunocytochemistry, hypoxia experiments, animal dissection, image analysis, and molecular biological experiments. There is an adjacent space of 780 sq ft solely for animal behavior.

Clinical: N/A

Animal: Animals will be maintained in the basements of the Elliman Building and Scott Hall, with Wayne State University's Department of Laboratory Animal Resources (DLAR), which is accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC). The staff of DLAR is comprised of 4 full-time veterinarians and more than 20 qualified technicians who are always on premises or on-call. DLAR provides food, bedding and cage changing services as well as procedure rooms.

Computer

1) Dr. Dore-Duffy has a Mac 9400 and HP Desk Jet printer. In her adjoining office for her administrative support, there is an iMac and HP Desk Jet Printer also. There is a Gateway PC with an HP Desk Jet printer and CanonScan 9900F flatbed scanner in the first lab and Mac G4 and Canon Laser Printer in the second lab. There is also a 500GB Seagate external drive for data backup. These computers can all access Wayne State University's graduate and medical library systems online and the internet via Ethernet and have the following software: Adobe Photoshop and Illustrator, Microsoft Office 2003, Endnote, ImageJ.
2) Dr. Gow has Pentium 3 and 4 (x2) and G4 Macintosh (x7) computers linked via ethernet to all other computers and peripheral devices in the Elliman building, the WSU library and the internet. Software: MacVector, Vector NTI, OpenLab, Volocity, Canvas, Photoshop, Illustrator, Filemaker Pro, SPSS, Prism, Microscoft Office 2001, Endnote. Hardware: flatbed scanner, Nikon CoolPix 950 digital camera, color and b/w laser printers, Fujix printer, CD/DVD writers, 1 terabyte hard disk storage.
3) Dr. Rafols has three (3) Dell Dimension 8400 PCs , one (1) Toshiba Portege laptop, and one (1) Epson Photo r100 printer. These computers can all access Wayne State University's graduate and medical library systems online and the internet via Ethernet and have Microsoft Office 2003, Endnote, ImageJ, SPSS, Adobe Photoshop software.

Office

1) Dr. Dore-Duffy has a separate office and administrative support, complemented by a research coordinator shared by the Neurology Department and an additional combined 200 sq. feet of space for meetings is available on the first and second floors of Elliman.
2) Dr. Gow has 100 sq. ft of office space with 2 desks and chairs, telephone, filing cabinets. Secretarial and administrative support is available through the CMMG, across the road

at Scott Hall, and the Dept of Neurology, one floor above the P.I.s lab. Desks and computers are available for personnel in the lab, and an additional combined 200 sq. feet of space for meetings is available on the first and second floors of Elliman.

3) Dr. Rafols and Dr. Kreipke each have a separate office with a shared a common area, which totals 900 sq ft. There are two (2) technicians and an administrative assistant to assist with manuscript preparation and submission. There is additional administrative support through the Anatomy and Cell Biology Department.

Other

1) Shared equipment (in Elliman, for Drs. Dore-Duffy and Gow) is comprised of MilliQ water purification system, autoclaves, ice machines, Nikon confocal microscopy facility, Viral Vector Core, microPET imaging laboratory (for animals), lyophilizer. 4°C walk-in cold room. The 2nd and 3rd floors of the Elliman building have a total of 6 common equipment rooms housing autoclaves, ice makers, dry ice, liquid nitrogen, 37°C controlled temperature cabinets; New Brunswick shakers for bacterial growth; 4 low and 3 high speed centrifuges; GSA/SS34/SW50 and 80Ti rotors; X-ray film processor; photography darkroom; in situ darkrooms; 2 scintillation counters; 2 gel dryers with speed-vacs; a UV/VIS recording spectrophotometer; fax machine; 2 photocopiers; 3 Leica fluorescence microscopes equipped with film cameras for data recording, Opticon PCT-200 cycler equipped with CTD-3200 detector, Molecular Dynamics Storm 860 Phosphoimager (in Scott Hall).
2) Shared equipment (in Scott Hall, for Dr. Rafols) for the Anatomy and Cell Biology Department Core resources is comprised of three (3) cryostats and additional (-80) freezers. The MRI center adjacent to Scott Hall and Elliman houses the 4.7T MRI needed to facilitate CBF measurements.

Major Equipment

1) Dore-Duffy labs – GeneAmp PCR System 9700, refrigerators, (-80) freezer, Micron cryostat, laminar flow fume hood, (2) bio-safety cabinet, Waters HPLC with detectors (Dual λ Absorbance, Multi λ Fluorescence), Sorvall benchtop centrifuges (GLC-4 and Microspin 12S), Sorvall RC 5B Plus Centrifuge, ELX-800 Bio-Tek ELISA reader, electrophoresis equipment (DNA and protein), Beckman scintillation counter, (2) BioSpherix normobaric hypoxia animal chambers each with ProOx 110 oxygen controllers, Axiovert 135 tissue culture microscope, (2) CO2 incubators.
2) Gow labs – Treadmill (rodent size) with analytical TreadScan software, Sorvall M120 SE ultracentrifuge with rotors, Nikon SMZ-10 and SMZ-1500 dissecting microscopes with Sony catseye CCD camera. Leica DMRA2 microscope with 10, 20, 40, 63 100x Plan Apo lenses, motorized Z stage, spinning disk laser confocal with Argon and HeNe lasers, Hamamatsu Orca ER camera and OpenLab morphometry software for histochemistry and immunofluorescence. Reichart ultracut R and paraffin microtomes, vibratome with cooling coil for EM and light microscopy, 2 diamond knives, phosphoimager cassette, 4°C, -20°C, -80°C, liquid N2 storage, RO/millipore water. Behavioral testing equipment for rotarod, ABR, DPOAE, ASSR and auditory gating.
3) Rafols – Treadmills (rodent size), AccuScan radial arm maze, AxioVert microscope with camera (AxioCam camera) and software (AxioVision/Plan), (-80) freezer, two (2) refrigerators, dessicator.

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. * First Name: Paula Middle Name:
* Last Name: Dore-Duffy Suffix: Ph.D.
Position/Title: Professor Department: Neurology
Organization Name: Wayne State University Division:
* Street1: 421 East Canfield
Street2: 3126 Elliman Building
* City: Detroit County: Wayne
* State: MI: Michigan Province:
* Country: USA: UNITED STATES * Zip / Postal Code: 48201
* Phone Number: 313-577-0354 Fax Number: 313-577-7552
* E-Mail: pdduffy@med.wayne.edu

Credential, e.g., agency login: aa0895

*** Project Role:** PD/PI **Other Project Role Category:**

***Attach Biographical Sketch** 1234-PDD BioSk.pdf Add Attachment | Delete Attachment | View Attachment

Attach Current & Pending Support Add Attachment | Delete Attachment | View Attachment

PROFILE - Senior/Key Person 1

Prefix: Dr. * First Name: Jose Middle Name:
* Last Name: Rafols Suffix: PhD
Position/Title: Professor Department: Anatomy and Cell Biology
Organization Name: Wayne State University Division:
* Street1: 540 Canfield Avenue
Street2: 9312 Scott Hall
* City: Detroit County: Wayne
* State: MI: Michigan Province:
* Country: USA: UNITED STATES * Zip / Postal Code: 48201
* Phone Number: 313-577-1049 Fax Number: 313-577-3125
* E-Mail: jrafols@med.wayne.edu

Credential, e.g., agency login: aa3302

*** Project Role:** Other (Specify) **Other Project Role Category:** Co-Principle Investigator

***Attach Biographical Sketch** 1235-rafols Biosk.pdf Add Attachment | Delete Attachment | View Attachment

Attach Current & Pending Support Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Senior/Key Person 2

Prefix: Dr. * First Name: Christian Middle Name:
* Last Name: Kreipke Suffix: PhD
Position/Title: Assistant Professor - Research Department: Anatomy and Cell Biology
Organization Name: Wayne State University Division:
* Street1: 540 Canfield Avenue
Street2: 9312 Scott Hall
* City: Detroit County: Wayne
* State: MI: Michigan Province:
* Country: USA: UNITED STATES * Zip / Postal Code: 48201
* Phone Number: 313-577-1049 Fax Number: 313-577-3125
* E-Mail: ckreipke@med.wayne.edu

Credential, e.g., agency login: aa5930

*** Project Role:** Other (Specify) **Other Project Role Category:** Co-Principle Investigator

***Attach Biographical Sketch** 1236-Kreipke biosk.pdf Add Attachment Delete Attachment View Attachment

Attach Current & Pending Support Add Attachment Delete Attachment View Attachment

PROFILE - Senior/Key Person 3

Prefix: Dr. * First Name: Alexander Middle Name:
* Last Name: Gow Suffix: PhD
Position/Title: Associate Professor Department: CMMG
Organization Name: Wayne State University Division:
* Street1: 540 East Canfield Avenue
Street2: 3126 Scott Hall
* City: Detroit County: Wayne
* State: MI: Michigan Province:
* Country: USA: UNITED STATES * Zip / Postal Code: 48201
* Phone Number: 313-577-9401 Fax Number:
* E-Mail: agow@med.wayne.edu

Credential, e.g., agency login: ai7965

*** Project Role:** Other (Specify) **Other Project Role Category:** Co-Principle Investigator

***Attach Biographical Sketch** 1237-Gow biosketch june 09.pd Add Attachment Delete Attachment View Attachment

Attach Current & Pending Support Add Attachment Delete Attachment View Attachment

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Senior/Key Person 4

Prefix: Dr. * First Name: Joseph Middle Name:

* Last Name: LaManna Suffix: PhD

Position/Title: Professor Department: Physiology & Biophysics

Organization Name: Case Western Reserve University Division: SOM

* Street1: 10900 Euclid Avenue

Street2: BRB - Room 525

* City: Cleveland County:

* State: OH: Ohio Province:

* Country: USA: UNITED STATES * Zip / Postal Code: 44106-4938

* Phone Number: 216-368-1112 Fax Number: 216-368-1144

* E-Mail: joseph.lamanna@case.edu

Credential, e.g., agency login: LAMANNA

*** Project Role:** Consultant **Other Project Role Category:**

***Attach Biographical Sketch** 1238-JCL biosk.pdf Add Attachment | Delete Attachment | View Attachment

Attach Current & Pending Support Add Attachment | Delete Attachment | View Attachment

ADDITIONAL SENIOR/KEY PERSON PROFILE(S) Add Attachment | Delete Attachment | View Attachment

Additional Biographical Sketch(es) (Senior/Key Person) Add Attachment | Delete Attachment | View Attachment

Additional Current and Pending Support(s) Add Attachment | Delete Attachment | View Attachment

OMB Number: 4040-0001
Expiration Date: 04/30/2008

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Paula Dore-Duffy, Ph.D.	Professor of Neurology
eRA COMMONS USER NAME aa0895	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Simmons College, Boston, MA	B.S.	1972	Biology
Baylor College of Medicine, Houston, TX	--	1973	Virology
Louisiana State University, School of Medicine, New Orleans, LA	Ph.D	1978	Microbiol. / Immunol.

A. Positions and Honors.

Positions and Employment

1979-1982 Assistant Professor, Neurology and Medicine, University of Connecticut School of Medicine, Farmington, CT
1982-1988 Associate Professor, Medicine, University of Connecticut School of Medicine, Farmington, CT
1982-1988 Associate Professor, Neurology, University of Connecticut School of Medicine, Farmington, CT
1982-1988 Director, UCHC MS Center, University of Connecticut School of Medicine, Farmington, CT
1977 Tenure, University of Connecticut School of Medicine, Farmington, CT
1984-1988 Co-Director, Neuroscience Graduate Program, University of Connecticut School of Medicine, Farmington, CT
1988-Pres Co-Director, Multiple Sclerosis Center, Wayne State University School of Medicine Detroit, MI
1989-Pres Chief, Division of Neuroimmunology, Department of Neurology, Wayne State University School of Medicine Detroit, MI
1988-Pres Professor, Neurology, Wayne State University School of Medicine Detroit, MI
1988-Pres Associate Professor, Immunology and Microbiology, Wayne State University School of Medicine, Detroit, MI
1996-1998 Co-Director, Detroit Neurotrauma Center, Wayne State University Detroit, MI

Other Experience and Professional Memberships

National Institutes of Health, Study Section Reviewer, BINP, 2005-2007
American Heart Foundation, Reviewer, 2007
Department of Defense Neurobiology Committee A, Member, 2007
Program Committee, Winter Conference on Brain Research 2007
American Neurological Association
American Academy of Neurology
The American Association of Immunologists (AAI)
Society for Neuroscience
American Society of Neurochemistry
The Royal Society of Medicine, newly appointed – 2008
National Neurotrauma Society
International Society for Blood Flow & Metabolism
International Brain Barrier Society

Honors / Awards

2008	Academy of Scholars, Wayne State University
2007	Faculty Excellence in Research Award, Wayne State University School of Medicine
1998	Deputy Editor, Journal of Neurological Sciences
1998	NIH Study Section, NB4
1996	Gershenson Distinguished Faculty Fellow
1994-Pres	Editorial Boards: Neurology, Microvascular Research, Journal of Experimental Microbiology and Immunology, Journal of Clinical Pharmacology
1988-1992	National Institute of Health Study Section (NSPA)
1985-1986	Visiting summer scientist Mount Desert Island Biological Labs, Salisbury Cove, Maine
1985	Kroc Foundation Endowment $50,000 to establish a yearly MS Symposium
1980	Multiple Sclerosis Society Bursar to the 4th International Congress of Immunology in Paris
1979	Outstanding Young Women Scientist of America
1977-1978	National Multiple Sclerosis Society Fellowship Award

B. Selected peer-reviewed publications (in chronological order).

Dore-Duffy, P., Donovan. C. and Todd, R.F. III. Monocyte Activation Associated Antigen MO3e in MS. Neurology. 42:1609-1615, 1992.

Couldwell, W.T., Yong, VE., Dore-Duffy, P., Freedman, M.S. and Antel, J.P. Production of Soluble Autocrine inhibitory factors by human glioma cell lines. J. Neurol. Sci. 110:178-185, 1992.

Washington, R., Burton J., Todd, R.F., III, Dragovic, L., and Dore-Duffy, P. Expression of immunologically Relevant Endothelial Cell Activation Antigens of Isolated CNS Microvessels from Patients with MS. Evidence for Focal Activation o f Vascular Endothelium. Ann. Neurol. 35:89-97, 1994.

Washington, R. and Dore-Duffy, P. Role of Cytodkeletal Elements in Expression of Monocyte Urokinase Plasminogen Activator Receptor, Activation-Associated Antigen Mo3. Clin. Diag. Lab. Immunol. 1(6): 714-721, 1994.

Dore-Duffy, P., Balabanov, R., Washington, R., and Swanborg, R.H. Transforming Growth Factor Beta-1 Inhibits CNS Endothelial Cell Activation. Mol. Chem. Neuropath. 3:161-175, 1994

Dore-Duffy, P., Washington, R., and Balabanov, R., Cytokine-mediated activation of cultured CNS microvessel: A system for examining antigenic modulation of CNS EC and evidence for long-term expression of adhesion protein E-selectin. J. Cereb .Blood Flow Metab. 14:837-884, 1994.

Dore-Duffy, P., Newman, W., Balabanov, R., Lisak, T.P., Rothlien, R. and Peterson, M. Evaluation of circulating soluble adhesion proteins in CSF and serum of patients with multiple sclerosis. Correlation with clinical activity. Ann. Neurol. 37(1):55-62, 1995.

Dore-Duffy, P., Balabanov, R., and Swanborg, R.H. The recovery phase of acute EAE in rats corresponds to development of endothelial cell unresponsiveness. J Neuroscience Research. 44:223-234, 1996.

Dore-Duffy, P., Balabanov, R. and Washington, R. Recovery from acute experimental autoimmune encephalomyelitis (EAE) characterized by endothelial cell unresponsiveness cytokines and pericytes activation. Biology and Physiology of the Blood-Brain Barrier. 57:347-351, 1996.

Washington, R., Becher, B., Balabanov, R. Antel, J., and Dore-Duffy, P. Expression of the Activation Marker Urokinase Plasminogen-Activator Receptor in Cultured Human Central Nervous systems Microglia. J. Neurosci Res. 45:392-399, 1996.

Balabanov, R., Beaumont, T. and Dore-Duffy, P. Role of Central Nervous System Microvascular pericytes in Activation of Antigen-Primed Splenic T-Lymphocytes. J. Neuroscience Res. 55:578-587, 1999.

Dore-Duffy, P., Balabanov, R., Beaumont, T., Hritz, M.A. Harik, S.I. and LaManna J.C. Endothelial activation Following Prolonged Hypobaric Hypoxia. Microvas. Res. 57:75,85 , 1999

Dore-Duffy, P., Balabanov, R., Owens, S., and Rafols, J. Migration of CNS microvascular pericytes

following traumatic brain injury. Microvas Res. 60:55-69, 2000.

Balabanov, R., Lisak, D., Beaumont, T., Lisak, R.P., Dore-Duffy, P. Expression of urokinase plasminogen activator receptor on monocytes from patients with relapsing-remitting multiple sclerosis: effect of glatiramer acetate (copolymer 1).. Clin. Diagn. Lab. Immunol. 8:1196-203, 2001.

Balabanov, R., Goldman, H., Murphy, S., Pellizon, G., Owen, C., Dore-Duffy, P., Endothelial cell Following Moderate Traumatic Brain Injury. Neurological Res. 23:175-182, 2001.

Balabanov, R., Dore-Duffy, P., The CNS microvascular pericyte response to hypoxia. Neurotrauma 19:1331, 2002.

Wagnerova, J., Cervenakova, L., Balabanov, R., and Dore-Duffy, P., Cytokine Regulation of E-Selectin in Rat CNS microvascular Endothelial Cells: Differential Response of CNS and Non-CNS Vessels. J Neurological Sciences 195:51-62, 2002

Katychev, A., Wang X., Duffy A., Troutner, A., and Dore-Duffy, P. Glucocorticoid Apoptosis in CNS microvascular pericytes. Dev. Neurosci. 25(6):436-46, 2003.

Dore-Duffy, P. Isolation of cerebral microvascular pericytes. Methods in Mol. Med. 89:375-382, 2003.

Dore-Duffy, P, Balabanov R, Wang X, Beaumont T. Pericyte release of cyclopentenone prostaglandins in response to hypoxia. Microvascular Research 35; 215-226 2005.

Katychev, A., Wang, X, and Dore-Duffy, P.,. Characterization of CNS microvascular pericytes and endothelial cells exhibit multipotential stem cell activity. J Cereb Blood Flow Metab. 26:613-624, 2006.

Dore-Duffy, P and LaManna, JC. Physiological Angiodynamics in the brain and Redox signaling. Antioxidants and Redox Signaling 9: 1363-1371, 2007.

Dore-Duffy, P., Wang, X., Mehedi, A., Kreipke, W., and Rafols, J. Differential expression of capillary VEGF isoforms following traumatic brain injury. Neurological Res. 29: 395-403, 2007.

Milner, R., Hung, S., Erokwu, B., Dore-Duffy, P., LaManna, J., and del Zoppo, G. Increased expression of fibronectin and the $\alpha 5\beta 1$ integrin in angiogenic cerebral blood vessels of mice subject to hypobaric hypoxia. Mol Cell Neurosci. 38: 43-52, 2008.

Dore-Duffy, P. Pericytes: Pluripotent cells of the blood brain barrier. Curr Pharm Des. 14: 1581-1593, 2008.

Del Zoppo, G., Hung, S., Wang, X., Dore-Duffy, P. Cathepsin L expression within the neurovascular unit. (submitted).

Dore-Duffy, P., Wang, X., Mehedi, A., LaManna JC. Coordinated capillary expression of TNF superfamily member TWEAK following exposure to low oxygen is important in physiological adaptive angiogenesis. (submitted).

Dore-Duffy, P., Cleary, K. "Morphology and Properties of the Neurovascular Unit: The Pericyte", *in* The Blood-Barrier and Other Neural Barriers: Biology and Research Protocols, Ed: S. Nag, Humana Press Inc, Totowa (submitted).

Mehedi, A., Wang, S, Gow, A., Dore-Duffy, P. Conditionally immortalized CNS capillary pericytes are pluripotent. (submitted).

Dore-Duffy, P., Mehedi, A., Wang, X., Trotter, R. Pericyte Differentiation: generalization of neurospheres from isolated capillaries. (In preparation).

Dore-Duffy, P., Mehedi, A., Wang, X. Mouse CNS Immortalized Pericytes (IMPs) differentiation along the neural lineage. (In preparation).

Puckett, J., Cleary, K., Dore-Duffy, P. Pericytes and stroke. (in preparation)

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Jose A. Rafols	Professor
eRA COMMONS USER NAME	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Illinois Benedictine, Lisle, IL	B.S.	1965	Biology
University of Kansas, Kansas City, KS	Ph.D.	1969	Anatomy
S. Ramon Y Cajal Institute, CSIC, Madrid, Spain	Post Doc	1970	Neuroanatomy

A. Positions and Honors

Positions and Employment

1969-1970	Instructor, Dept. of Anatomy/Cell Biology, Wayne State University, School of Medicine
1970	NIH Postdoctoral trainee at S. Ramon Y Cajal Institute, CSIC, Madrid, Spain
1971-1973	Asst. Professor, Dept. of Anatomy/Cell Biology, Wayne State University, School of Medicine
1973-1989	Assoc. Professor, Dept. of Anatomy/Cell Biology, Wayne State University, School of Medicine
1989-present	Professor, Dept. of Anatomy/Cell Biology, Wayne State University, School of Medicine
1994-present	Dir., Morphology and Imaging Core, Neurotrauma Center, Wayne State University, School of Medicine

Honors

DHHS/PHS/NIH Study Section Member (full member), Neurological Disorder Program Project Review A Committee (NSP-term) 7/1/90-6/30/94.
Chairman, Site visit, The Johns Hopkins University, Baltimore, MD; "Disorders of aging neuro-transmitter systems and neurotrophins", December 15-17, 1991.
Member, National Institutes of Health Reviewers Reserve (NRR), for term 7/1/94-6/30/98.
Member, American Heart Association National Study Committee, Brain Review Committee, for term 7/96-6/99.

B. Selected peer-reviewed publications (past five years)

Petrov T, Page AB, Owen C, Rafols JA. 2000 Expression of the inducible nitric oxide synthase in distince cellular types after traumatic brain injury. Acta Neuropathol 100:196-204.
Dore-Duffy P, Owen C, Bahabanov R, Murphy S, Rafols JA. 2000 Pericyte response to traumatic brain injury (TBI): Elongation and migration from the microvascular wall. Microvascular Res 60:55-69.
White BC, Sullivan JM, DeGracia DJ, O'Neill BJ, Neumar RW, Grossman LI, Rafols JA, Krause GS. 2000 Brain ischemia and reperfusion: Molecular mechanisms of neuronal injury. J Neurolog Sci 179:1-33.
Owen CR, Lipinski C, Page AB, White BC, Sullivan JM, DeGracia DJ, Rafols JA, Krause GS. 2001 Characterization of the eIF2ά-assocated protein p67 during brain ischemia and reperfusion. In Maturation Phenomen Cerebral Ischemia IV. Springer-Verlag, Berlin-Heidelberg pp. 19-24.
Petrov T, Underwood W, Alousi S, Own C, Rafols JA. 2001 Upregulation of iNOS and eIF2άP expression is paralleled by suppression of protein synthesis in the hypothalamus following trauma to the brain. J Neurotrauma 18:799-812.
Petrov T and Rafols JA. 2001 Acute alterations of endothelin-1 and iNOS expression and control of the brain microcirculation after head trauma. Neurol Res 23:138-143.
Balabanov R, Goldman R, Murphy S, Pellizon G, Own C, Rafols JA, Dore-Duffy P. 2001 Endothelial cell activation following moderate trauamatic brain injury. Neurol Res 23:175-182.
Barnes M, Lapanosky K, Rafols JA, Lawson DM, Dunbar JC. 2001 GnRH release is decreased in the absence of nitric oxide. PSEBM 226:701-706.
Petrov T, Steiner J, Braun B, Rafols JA. 2002 Sources of endothelin-1 in hippocampus and cortex following traumatic brain injury. Neurosci 115:275-283.

Barnes MJ, Lapanowski K, Rafols JA, Lawson DM, Dunbar JC. 2002 Chronic nitric oxide deficiency is associated with altered leutinizing hormone and follicle-stimulating hormone release in ovariectomized rats. PSEBM 233:817-822.

Ding Y, Li J, Phillis JW, Rafols JA, Diaz FG. 2002 Preperfusion infusion into ischemic territory reduces inflammatory injury after transient middle cerebral artery occlusion in rat. Stroke 33:2492-2498.

Ding Y, Li J, Lai Q, Azam S, Rafols JA, Diaz FG. 2002 Functional improvement after motor training is correlated with synaptic plasticity in rat thalamus. Neurol Res 24:829-836.

Petrov T, Rafols JA, Alousi SS, Kupsky WJ, Johnson R, Shah J, Shah A, Watson C. 2003 Cellular compartmentalization of Phosphorylated eIF2 alpha and neuronal NOS in human temporal lobe Epilepsy with hippocampal sclerosis. J Neurol Sci 209:31-39.

Ding Y, Li J, Clark J, Diaz FG, Rafols JA. 2003 Synaptic plasticity in thalamic nuclei enhanced by motor skill training in rat with transient middle cerebral artery occlusion. Neurol Res 25:189-194.

Ding Y, Li J, Rafols JA, Clark J, Phillis JW, Diaz FG. 2003 Preischemic motor exercise reduces ischemia/reprofusion injury in rats that correlates with regional angiogenesis and cellular expression of neurotrophin. Stroke 34:240-241.

Barnes MJ, Lapanowski K, Conley A, Rafols JA, Catherine KL, Dunbar JC. 2003 High fat feeding is associated with increased blood pressure, sympathetic nerve activity and hypothalamic mu opiod receptrs. Brain Res Bull 61:511-519.

Britton M, Rafols JA, Alousi S, Dunbar JC. 2003 The effects of middle cerebral artery occlusion on central nervous system apoptotic events in normal and diabetic rats. Experimental Diab Res 4:13-20.

Ding Y, Li J, Lai Q, Rafols JA, Luan X, Clark J, Diaz F. 2004 Motor balance and coordination training enhances functional outcome in rat with transient middle cerebral artery occlusion. Neuroscience 123:667-674.

Steiner J, Rafols D, Park H, Katar MS, Rafols JA, Petrov T. 2004 Attenuation of inOS mRNA exacerbates hypoperfusion and upregulates endothelin-1 expression in hippocampus and cortex after brain trauma. Nitric Oxide 10:162-169.

Rafols D, Steiner J, Rafols JA, Petrov T. 2004 Coexpression of iNOS and endothelin-1 mRNAs in specific cell types following traumatic brain injury. Neurosci letters 362:154-157.

Li J, Luan X, Clark J, Rafols JA, Diaz FG, Ding Y. 2004 Regional brain cooling induced by local saline infusion into ischemic territory produced a long-term neuroprotection in ischemic rats using a behavioral assessment. Neurol Res 26:677-683.

Ding Y, Li J, Rafols JA, Clark JC, McAllister JPII, Diaz FG, Guthikonda M, Ding Y. 2004 Exercise-induced angiogenic factors and reduction in ischemia/reperfusion injury. Current Neurovasc Res 1:411-420.

Dunbar J, Lapanowski K, Barnes M, Rafols JA. 2005 Hypothalamic agouti-related protein immunoreactivity in food-restricted, obese, and insulin-treated animals: evidence for glia cell localization. Exptl Neur 191:184-192.

Ding Y, Ding Y, Yojng C, Luan X, Li J, Rafols JA, Phillis JW, Calrck JC. 2005 Exercise pre-conditioning reduces inflammatory injury in ischemic rats during reperfusion. Acta neuropathol 109:237-246.

Kayali F, Montie H, Rafols JA, DeGracia DJ. 2005 Prolonged translation arrest in reperfused hippocampal Ca1 is mediated by Stress granules. Neurosci 134:1223-1245.

Kreipke C, Rafols J, Petrov T. 2005. Transcriptional and translational mechanisms for the reciprocal control of iNOS and endothelin 1 expression in brain microvessels after traumatic brain injury (TBI). Journal of Cerebral Blood Flow and Metabolism 25, S191.

Kreipke CW, Morgan N, Petrov T, Rafols J. 2006. Calponin and caldesmon cellular domains in reacting microvessels following traumatic brain injury. Microvascular Research. 71:197-204.

Kreipke CW, Morgan R, Petrov T, Rafols JA. 2007. Subcellular Redistribution of Calponin Underlies Sustained Vascular Contractility Following Traumatic Brain Injury. Neurol Res. 29:604-609.

Rafols J., Kreipke C, Petrov T. 2007. Alterations in Cerebral Cortex Microvessels and the Microcirculation in a Rat Model of Traumatic Brain Injury: a Correlative EM and Laser Doppler Flowmetry Study. Neurol Res 29:339-347.

Rafols J, Morgan R, Kallikuri S, Kreipke C. 2007. Extent of nerve cell injury in Marmarou's model compared to other brain trauma models. Neurol Res 29:348-355.

Degracia D, Kreipke C, Kayali F, Rafols JA. 2007. Brain endothelial HSP-70 stress response coincides with endothelial and pericyte death after brain trauma. Neurol Res 29:356-361.

Kallukuri S, Kreipke C, Rossi NF., Rafols JA, Petrov T. 2007. Spatial alterations in endothelin receptor expression are temporally associated with the altered microcirculation after brain trauma Endothelin receptor localization following traumatic brain injury. Neurol Res 29:362-368.
Kreipke C, Morgan R, Roberts G, Bagchi M, Rafols JA. 2007. Calponin phosphorylation in cerebral cortex microvessels mediates sustained vasoconstriction after brain trauma. Neurol Res 29:369-374.
Morgan R, Kreipke C, Robert G, Bagchi M, Rafols J. 2007. Neovascularization following traumatic brain injury: possible evidence for both angiogenesis and vasculogenesis. Neurol Res 29:375-381.
Kreipke CW, Morgan R, Kallakuri S, Rafols JA. 2007. Behavioral pre-conditioning enhances angiogenesis and cognitive outcome after brain trauma. Neurol Res. 29:388-94.
Dore-Duffy P, Kreipke C, Rafols JA. 2007. Differential expression of capillary VEGF isoforms following traumatic brain injury. Neurol Res 29:395-403.

Earlier Pertinent Publications

Rafols JA, Getchell TV. 1983 Morphological relations between the receptor neurons, sustentacular cells and Schwann cells in the olfactory mucosa of the salamander. Anat Rec 206:87-101.
Goshgarian HG, Rafols JA. 1984 The ultrastructural and synaptic architecture of phrenic motor neurons in the spinal cord of the adult rat. J Neurocytol 13:85-109.
Getchell ML, Rafols JA, Getchell TV. 1984 Histological and histochemical studies of the secretory components of the salamander olfactory mucosa: Effects of isoproterenol and olfactory nerve section. Anat Rec 208:553-565.
Rafols JA, Goshgarian H. 1985 Spinal tanycytes in the adult rat: A correlative Golgi—gold toning study. Anat Rec 211:75-86.
Rafols JA, Aronin N, DiFiglia M. 1986 A Golgi study of the monkey paraventricular nucleus: Neuronal types, afferent and efferent fibers. J comp Neur 257:585-613.
Rafols JA. 1986 Ependymal tanycytes of the ventricular system in vertebrates. In: Astrocytes: Development, Morphology and Regional Specialization of Astrocytes, vol. 1. Cellular Neurobiology: A series. S. Federoff and A. Vernadakis, Academic Press, Inc. Orlando, pp. 131-148.
Rafols JA and McNeill TH. 1987 Age-related dendritic changes of spiny and aspiny neurons in the rodent striatum. In the Basal Ganglia II: Structure and Funciton-Current Concepts. MB Carpenter and A JAyaraman (ed.s) Adv Behav Biol, vol 32, Plenum, New York, pp. 227-239.
McNeill TH, Brown SA, Rafols JA, Shoulson I. 1988 Regression of striatal dendrites in Parkinson's Disease. Brain Res 455:148-152.
Rafols JA, Cheng HW, McNeill TH. 1989 Golgi study of the mouse striatum: Age-related dendritic changes in different neuronal populations. J Comp Neur 279:212-227.
Goshgarian HC, YU XJ, Rafols JA. 1989 Neuronal and glial changes in the rat phrenic nucleus occurring within hours after spinal cord injury. J Comp Neur 284:519-533.
Ma TP, Cheng HW, Czech JA, Rafols JA. 1990 The intermediate and deep layers of the macaque superior colliculus: A Golgi study. J comp Neur 294:2-20.
McNeill TH, Koek LL, Brown SA, Rafols JA. 1991 Quantitative analysis of age-related dendritic changes in medium spiny I (MSI) striatal neurons of C57BL/6N mice. Neurobiol Aging 11:21-31.
Ma TP, Hu J, Anavi Y, Rafols JA. 1992 organization of the zona incerta in the macaque: Nissl and Golgi study. J Comp Neur 320:273-290.
White B, Daya A, DeGracia DJ, Krause G, Rafols JA. 1993 Fluorescent histochemical localization of lipid peroxidation during brain reperfusion following cardiac arrest. Acta Neuropath (Berlin) 86:1-90.
Crossland W, Hu X-J, Rafols JA. 1994 A morphological study of the rostral interstial nucleus of the medial longitude fasciculus in the monkey, *macaca mulatta*, by Nissl, Golgi, and Computer reconstruction methods. J comp Neurol 1:1-17.
Rafols JA, Daya AM, Krause GS, Neumar RW, White BC. 1995 Global brain ischemia and reperfusion: Golgi apparatus untrastructure in neurons selectively vulnerable ot death. Acta Neuropathol 90:17-30.
Rafols JA, Own C, Murphy S, Dore-Duffy P. 1995 Pericyte response following traumatic brain injury: Migration pericytes from CNS microvessels and apoptosis. J Neurotrauma 12:988 (vol 5).
O'Neil BJ, Krause LI, Grossman LI, Grunberger G, Rafols JA, DeGracia DJ, Newar BR, Tiffany BR, White BC. 1995 Global ischemia and reperfusion by cardiac arrest and resuscitation: Mechanisms leading to death of vulnerable neurons and a fundamental basis for therapeutic approaches. Cardiac Arrest: The Science of Practice of Resuscitation Medicine. Paradis, Halpern, and Nowak (eds.) Williams and Wilkins, Ch.5, pp. 84.
Ma TP, Lynch JC, Donahoe DK, Attallah H, Rafols JA. 1996 Organization of the medial pulvinar nucleus in the macaque. J Comp Neurol Anat Rec 250:220-237.
Page AB, Krause GS, Rafols JA. 1996 Differential expression of iNOS in rat cortex following trauma. Soc Neurosci (Abstracts) 22:2157.

Lenzi T, Raols JA. 1996 Reperfusion-induced changes in a nine-vessel occlusion model of ischemia. Soc NEurosci (Abstracts) 22:2157.
Neumar RW, Alousi SS, White BC, Rafols JA. 1996 Immunogold labeling of CaMKII in hippocampal neurons during global ischemia. Soc NEurosci (Abstracts) 22:1896.
Folkerts MM, Berman Wang G, Murphy S, Rafols JA, Muizelaar JP. 1996 Behavior morphological and electrophysiological effects of diffuse axonal injury in rats. J Neurotrauma 13:610.
Folkerts MM, Berman RF, Muizelaar JP, Rafols JA. 1998 Disruption of MAP2 immunostaining in rat hippocampus traumatic brain injury. J Neurotrauma 15:349-363.
Sullivan JM, Alousi SS, Kikade KR, Rafols JA, Krause GS, White BC. 1998 Insulin induces dephosphorylation of eIF2alpha(P) and restores protein synthesis in vulnerable hippocampal neurons following transient ischemia. J Cereb Blood Flow Metab 19:1010-1019.
O'Neil BJ, McKeown TR, DeGracia DJ, Alousi SS, Rafols JA, White BC. 1998 Cell death, calcium mobilization and immunostaining for phosphorylation eukaryotic initiation factor 2alpha in nueronally-differentiated NB-cells: Arachidonate and radical-mediated injury mechanisms. Resuscitation 41:71-83.
Goldstein EN, Own CR, White BC, Rafols JA. 1999 Ultrastructural localization of phosphorylated eIF2(alpha)P during brain reperfusion. Acta Neuropathol 98:493-505.
McKeown TR, Goldstein EN, Sullivan JM, Rafols JA, White BC, Krause GS. 1999 Nuclear localization and DNA binding properties of phosphorylated eIF2alpha (P) ub vulnerable hippocampal neurons during reperfusion. J Cereb Flow Metab 19:S510.
Rafols JA, Alousi SS, Owen CR, White BC, Sullivan JM. 1999 High doses of insulin do not prevent dephosphorylation of eIF2alpha(P), recovery of protein synthesis, and atrophy of hippocampal cA3 neurons during reperfusion. J Cereb Flow Metab 19:s511.
Petrov T, Own CR, Rafols JA. 1999 Differential synthesis of endothelin (ET-1) and nitric oxide (NO) in rat cereb microvessels following traumatic brain injury (TBI). Soc. Neurosci (Abstracts) 25:822.
Underwood BD, Lipinski CA, Rafols JA, Crossland WC, McAllister JP, Diaz FG, White BC. 1999 Effects of traumatic brain injury on phosphorylated eIF2alpha in the rat. Soc. Neurosci (abrstract) 25:820.

C. Ongoing Research Support

VA RR & D Award. Rossi (PI) 1/01/08-12/31/11
VA Rehabilitation
Role: CO-I (20% Effort)
"Conditioning, microvascular tone & rehabilitation post brain trauma"
Investigates the role of exercise in the control of microcirculation in a rat model of traumatic brain injury.

NIH-NINDS RO1 NS39860 06/01/04-05/31/09
Control of Microvascular tone in Traumatic Brain Injury.
The long term objective of this project is to investigate the effects of traumatic brain injury on the gene regulation and synthesis of molecules which effect contractility or relaxation of the smooth muscle cells in the wall of cerebral microvessels. The hypothesis being tested is that altered regulation of the genes that encode for endothelin receptors in endothelial cells, at different time points participate in the abnormal contractility of brain microvessels following trauma.
PI

NIH-NINDS RO1 NS044100 07/01/03-06/30/08
The Unfolded Protein Response after Brain Ischemia.
The proposal aims at investigating the unfolded-protein response as a mechanism of cell death in hippocampal CA1 neurons after brain ischemia/reperfusion.
Co-I

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Christian W. Kreipke	Assistant Professor, Research
eRA COMMONS USER NAME	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Wayne State University	B.A.	1995-1999	Anthropology
Wayne State University	M.A.	1999-2000	Medical Anthropology
Wayne State University, School of Medicine	Ph.D.	2000-2004	Neuroscience

A. Positions and Honors

01/97-05/97 Wayne State University, School of Medicine and Hutzel Hospital, Research Assistant, Bone Densitometry/Osteoporosis Project

09/97-09/99 Wayne State University, Institute for Information and Technology, Research Assistant, HIV/AIDS in Detroit Project

09/99-05/00 Wayne State University, Graduate Teaching Assistant, Department of Anthropology

05/00-09/00 Wayne State University, Adjunct Instructor, Department of Anthropology

09/00-08/04 Wayne State University, School of Medicine, Pre-Doctoral Research Assistant, National Institute of Drug Abuse T32 Training Grant

08/04-04/08 Wayne State University, School of Medicine, Research Associate, Dept. Anatomy and Cell Biology, Traumatic Brain Injury

04/08-present Wayne State University, School of Medicine, Research Scientist, Dept. Anatomy and Cell Biology

Other Experience and Professional Memberships

05/99-present Member, Phi Beta Kappa

02/00-present Member, Society for Applied Anthropology

02/00-present Member, Society for Medical Anthropology

05/01-present Member, Sigma Xi

05/01-present Member, New York Academy of Sciences

03/01-03/02 Society for Neuroscience Brain Awareness Week Committee, Wayne State University, Chair

05/02-present Member, Society for Neuroscience

05/02-05/04 Michigan Society for Neuroscience, Student Counselor

05/03 Michigan Society for Neuroscience Chapter Meeting coordinator

11/04-08/07 Sigma Xi, Wayne State Chapter, Executive Board Member

02/05-08/07 Wayne State Alumni Communications Committee, Committee Member

05/06-08/07 Sigma Xi, National, Associate Director, NorthCentral Region

03/07-present Member, International Society for Cerebral Blood Flow and Metabolism

02/07-present Chairman of the Board, Southfield Oncology Institute

08/07-present Sigma Xi, National, Acting Director, NorthCentral Region

02/08-present Full Member of The Royal Society

Honors

2002 Dean Thomas Asselin, M.D. Endowed Prize for Excellence in Psychiatry and Behavioral Neuroscience Research (Wayne State University School of Medicine)

2003 1st Place, Society for Neuroscience, MI Chapter, Poster Award

2006 Service Award For 2006 Sigma Xi National Conference

2007 Travel Award, Brain '07, Society for Cerebral Blood Flow and Metabolism

2007 Young Investigators Award, Endothelin 10, Endothelin

B. Peer-reviewed publications (in chronological order)

1. Kuhn DM, Sadidi M, Lu X, **Kreipke C**, Geddes T, Borges C, and Throck J. 2002. Peroxynitrite-Induced Nitration of Tyrosine Hydroxylase: Identification of Tyrosines 423, 428, and 432 as Sites of Modification by MALDI-TOF Mass Spectrometry and Tyrosine-Scanning Mutagenesis. Journal of Biological Chemistry 277:14336-14342.
2. **Kreipke CW**, Walker PD. 2004. NMDA receptor blockade attenuates locomotion elicited by intrastriatal dopamine D1-receptor stimulation. Synapse 53:25-32.
3. **Kreipke C**, Rafols J, Petrov T. 2005. Transcriptional and translational mechanisms for the reciprocal control of iNOS and endothelin 1 expression in brain microvessels after traumatic brain injury (TBI). Journal of Cerebral Blood Flow and Metabolism 25, S191.
4. **Kreipke CW,** Campbell BM, Walker PD. 2005. Failure of MK-801 to suppress D1 receptor-mediated induction of locomotor activity and striatal preprotachykinin mRNA expression in the dopamine-depleted rat. Neuroscience 137:505-517.
5. **Kreipke CW**, Morgan N, Petrov T, Rafols J. 2006. Calponin and caldesmon cellular domains in reacting microvessels following traumatic brain injury. Microvascular Research 71:197-204.
6. Shen Y, Kou Z, **Kreipke CW**, Petrov T, Hu J, Haacke EM. 2007. In vivo measurement of tissue damage, oxygen saturation changes and blood flow changes after experimental traumatic brain injury in rats using susceptibility-weighted imaging. Magn Reson Imaging 25:219-227.
7. **Kreipke CW**, Morgan R, Petrov T, Rafols JA. 2007. Subcellular Redistribution of Calponin Underlies Sustained Vascular Contractility Following Traumatic Brain Injury. Neurological Research 29:604-609.
8. Petrov T, **Kreipke CW**, Alilain W, Nantwi K. 2007. Differential Expression Adenosine A_1 and A_2 Receptor Protein Levels Following Upper Cervical (C2) Spinal Cord Hemisection In Adult Rats. Journal of Spinal Cord Medicine 30:331-337.
9. Rafols J., **Kreipke CW**, Petrov T. 2007. Alterations in Cerebral Cortex Microvessels and the Microcirculation in a Rat Model of Traumatic Brain Injury: a Correlative EM and Laser Doppler Flowmetry Study. Neurological Research 29:339-347.
10. Rafols J, Morgan R, Kallikuri S, **Kreipke CW**. 2007. Extent of nerve cell injury Marmarou's model compared to other brain trauma models. Neurological Research 29:348-355.
11. Degracia D, Kayali F, **Kreipke CW**, Rafols JA. 2007. Brain endothelial HSP-70 stress response coincides with endothelial and pericyte death after brain trauma. Neurological Research 29:356-361.

12. Kallukuri S, **Kreipke CW**, Rossi N., Rafols JA, Petrov T. 2007. Spatial alterations in endothelin receptor expression are temporally associated with the altered microcirculation after brain trauma. Neurological Research 29:362-368.
13. **Kreipke CW**, Morgan R, Roberts G, Bagchi M, Rafols JA. 2007. Calponin phosphorylation in cerebral cortex microvessels mediates sustained vasoconstriction after brain trauma. Neurological Research 29:369-374.
14. Morgan R, **Kreipke CW**, Roberts G, Bagchi M, Rafols J. 2007. Neovascularization following traumatic brain injury: Possible evidence for both angiogenesis and vasculogenesis. Neurological Research 29:375-381.
15. **Kreipke CW**, Morgan R, Kallikuri S, Rafols J. 2007. Behavioral preconditioning enhances angiogenesis and cognitive outcome following traumatic brain injury. Neurological Research 29:388-394.
16. Dore-Duffy P, Xeuqain W, Mehedi A, **Kreipke CW**, Rafols JA. 2007. Differential expression of capillary VEGF isoforms following traumatic brain injury. Neurological Research 29:395-403.
17. **Kreipke CW**, Petrov T, Rafols JA. 2007. Endothelin A receptor antagonism blocks calponin phosphorylation following brain trauma. J Cereb Blood Flow and Metab, 26, S191.
18. Morgan R, **Kreipke CW**, Rafols JA. 2007. VEGFR2 antagonism attenuates behavioral improvements on a radial arm maze following traumatic brain injury. J Cereb Blood Flow and Metab, 26, S192.
19. Huttemann M, Lee I, **Kreipke CW**, Petrov T. 2008. Suppression of iNOS prior to traumatic brain injury improves cytochrome oxidase activity and normalizes cellular energy levels. Neuroscience 151:148-151.
20. **Kreipke CW,** Schafer PC, Rafols JA. 2008. Endothelin receptor A antagonism ameliorates hypoperfusion and enhances cognitive outcome following traumatic brain injury. Brain Injury 22:S43.
21. Rafols JA, **Kreipke CW,** Kallakuri S. 2008. Upregulation of endothelin-1 receptors in neurons and brain microvessels coincides temporally with a dysfunctional microcirculation after traumatic brain injury. Brain Injury 22:S44.
22. Hoffman W, Artlett C, Zhang W, **Kreipke CW**, Passmore G, Rafols JA, Sima AA. (2008) Receptor for advanced glycation end products and neuronal deficit in the fatal brain edema of diabetic ketoacidosis. Brain Research 1238:154-62.
23. **Kreipke CW,** Rafols JA. (2009) Calponin control of cerebrovascular reactivity: Therapeutic implications in brain trauma. J Cell Mol Med 13(2):262-9.
24. **Kreipke CW**, Schafer PC, Michael D, Rafols JA. (in press). Endothelin receptors A and B are expressed in distinct cellular compartments of rat hippocampus following global ischemia: an immunocytochemical study. Can J Physio Pharm.
25. Hoffman WH, Stamatovic SM, Rafols JA, **Kreipke CW**, Andjelkovic AV. (in press). Inflammatory mediators and blood brain barrier disruption in fatal brain edema of diabetic ketoacidosis. Experimental Diabetes Research.
26. **Kreipke CW**, Schafer PC, Rossi NF, Rafols JA. (in press). Differential affects of Endothelin receptor-A and B antagonism on hypoperfusion following traumatic brain injury (TBI). Neurological Research.
27. Kallakuri S, **Kreipke CW**, Schafer PC, Schafer SM, Rafols JA. (in press) Brain cellular localization of endothelin receptor A and B in a rodent model of diffuse brain injury. Neuroscience.
28. Schafer PC, Schafer SM, **Kreipke CW.** (in press). Effects of light-dark cycle on motoric and cognitive activity: Implications for behavioral testing. Bio Behav Res.
29. **Kreipke CW,** Schafer PC, Schafer SM, Pirooz R, Angoa-Perez M, Rafols JA, Kuhn DM. (in press). Extent of hypoperfusion in a murine model of diffuse brain injury. J Neurotrauma.

30. Ding JY, **Kreipke CW**, Speirs S, Schafer PC, Schafer S, Rafols JA. (in press). Hypoxia inducible factor-1□ signaling in aquaporin upregulation after traumatic brain injury. Neuros Lett.
31. Ding JY, **Kreipke CW**, Speirs S, Schafer PC, Schafer S, Rafols JA. (in press). Synapse Loss Regulated by Matrix Metalloproteinases in Traumatic Brain Injury Is Associated with Hypoxia-Inducible Factor-1α Expression. Brain Research.
32. Sima A, Zhang W, **Kreipke CW**, Rafols JA, Hoffman WH. (in press). Effect of C-peptide on the innate immune responses in diabetic hippocampus. Diabetes.

C. Research Support

Ongoing Research Support

R01 NS39860 T. Rafols (PI) 3/10/04-4/30/09
NIH-NINDS
Role: CO-I (70% effort)
"Control of microvascular tone in traumatic brain injury"
Investigates the role of endothelin receptors in the control of the microcirculation in a rat model of traumatic brain injury. There is NO overlap between this project and the current proposal. Effort on this project will be reduced to 40% upon successful funding of the current grant.

VA RR & D Award. Rossi (PI) 1/01/08-12/31/11
VA Rehabilitation
Role: CO-I (30% Effort)
"Conditioning, microvascular tone & rehabilitation post brain trauma"
Investigates the role of exercise in the control of microcirculation in a rat model of traumatic brain injury. There is NO overlap between this project and the current proposal.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Alexander Gow	Associate Professor
eRA COMMONS USER NAME ai7965	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
NSWIT, Sydney, Australia	B.S.	1977-1979	Biochemistry
NSWIT, Sydney, Australia	M.S.	1980-1983	Neuroscience
Queensland University, Brisbane, Australia	Ph.D.	1984-1990	Biochemistry
Mount Sinai School of Medicine, New York	Postdoc	1990-1994	Mol. Cell. Neurosci.

A. PROFESSIONAL POSITIONS

1980-1983	Teaching Assistant	School of Life Sciences, NSWIT, Sydney, Australia
1983-1990	Research Assistant	Biochemistry Dept, Queensland University, Brisbane, Australia
1990-1994	Research Associate	Brookdale Center, Mount Sinai School of Medicine, New York
1995-2000	Assistant Professor	Brookdale Center, Mount Sinai School of Medicine, New York
2000-2004	Assistant Professor	Center for Molecular Medicine and Genetics/Pediatrics/Neurology, Wayne State University, Detroit, Michigan
8-19-04	Associate Professor	CMMG/Pediatrics/Neurology, Wayne State University, Detroit, MI.
8-19-04	Tenure	Pediatrics Dept, Wayne State University, Detroit, MI.

HONORS AND AWARDS

2002	5R01, NINDS, NS43783	4th percentile
2003	5R01, NIDCD, DC006262	9.5th percentile
2003	Full Member, Karmanos Cancer Institute	
2004	Tenured Associate Professor, Wayne State University, Detroit, MI 48201.	
2004	Research Excellence Award, School of Medicine, Wayne State University, Detroit, MI 48201.	
2005	Appointment to Board of Experts, Italian Committee for Research Evaluation (CIVR).	
2005 – 2007	Treasurer, Executive Committee of the Academic Senate, Wayne State University School of Medicine.	
2005	Member, Sigma Xi.	
2006 – 2007	Wayne State University Career Development Award.	
2007 – 2012	Co-Investigator, Center of Excellence Award, National Multiple Sclerosis Society.	

CERTIFICATION AND ORGANIZATIONS

1981-present, Australian Society for Biochemistry and Molecular Biology; 1995-present, American Society of Neurochemistry; 1997-present, American Society for Cell Biology; 1998-present, American Society for Neuroscience; 2000-2001, Chair, Young Investigators Award Committee, American Society for Neurochemistry; 2002-2005, Member of Council, American Society for Neurochemistry; 2003-2004, Member, Marion Kies Award Committee, American Society for Neurochemistry; 2004-present, Scientific Advisory Committee, Charles S. Mott Center, Wayne State University.

REVIEWER

Ad Hoc Reviewer (grants): 1998-present, Multiple Sclerosis Society of Canada; 1998-present, Multiple Sclerosis Society of Great Britain and Northern Ireland; 1997-1999, Human Frontiers Science Program; 1998-present, Italian Ministry for University and Research; 2002-present, United States-Israel Bi-national Science Foundation; 2001-present, Wellcome Trust, UK; 2001-present, National Science Foundation, USA; 2004-present, European Leukodystrophy Association.

Editorial Boards: Journal of Neuroscience Research.
Ad-Hoc Reviewer (journals): Journal of Neuroscience; Trends in Genetics; J. Neurochemistry; Molecular and Cellular Biology; J. Neuroscience Research; Developmental Neuroscience; International J. Dev. Neuroscience; J. Neuroscience Methods; Nature Genetics; EMBO J.; J. Cell Biology; Neuron.

B. SELECTED PUBLICATIONS:

Gow A, Winzor DJ, Smith R (1985) Pressure-induced dissociation of aggregates of myelin proteolipid protein. Biochim. Biophys. Acta 828, 383-386.

Gow A, Winzor DJ, Smith R (1987) Equilibrium binding of myristoyl lysophosphatidylcholine to bovine myelin basic protein: an example of ligand-mediated acceptor association. Biochemistry 26, 982-987.

Gow A, Smith R (1989) The thermodynamically stable state of myelin basic protein in aqueous solution is a flexible coil. Biochem. J. 257, 535-540.

Gow A, Auton W, Smith R (1990) Interactions between bovine myelin basic protein and zwitterionic lysophospholipids. Biochemistry 29, 1142-1147.

Gow A, Winzor DJ, Smith R (1990) Preferential ligand binding to multistate acceptor systems: the unexplored paradox of acceptor self-association that is ligand-mediated but detrimental to ligand binding. J. Theor. Biol. 145, 407-420.

Gow A, Friedrich Jr VL, Lazzarini RA (1992) Myelin basic protein gene contains separate enhancers for oligodendrocyte and Schwann cell expression. J. Cell Biol. 119, 605-616.

Friedrich Jr VL, Holstein G, Li X, **Gow A**, Kelley KA, Lazzarini RA (1993) Intracellular distribution of transgenic bacterial β-galactosidase in CNS neurons and neuroglia. J. Neurosci. Res. 36, 88-98.

Gow A, Friedrich Jr VL, Lazzarini RA (1994) Intracellular transport and sorting of the oligodendrocyte transmembrane proteolipid protein. J. Neurosci. Res. 37, 563-573.

Gow A, Friedrich Jr VL, Lazzarini RA (1994) Many naturally occurring mutations of myelin PLP impair its intracellular transport. J. Neurosci. Res. 37, 574-583.

Tosic M, **Gow A**, Dolivo M, Domanska-Janik K, Lazzarini RA, Matthieu J-M (1995) Proteolipid/DM20 proteins bearing the paralytic tremor mutation in peripheral nerves and transfected COS-7 cells. Neurochem. Res. 21, 423-430.

Gow A, Lazzarini RA (1996) A cellular mechanism governing the severity of Pelizaeus-Merzbacher disease. Nat. Genet. 13, 422-428.

Jaquet V, **Gow A**, Tosic M, Suchanek G, Breitschopf H, Lassmann H, Lazzarini RA, Matthieu J-M (1996) An antisense transgenic strategy to inhibit the myelin oligodendrocyte glycoprotein synthesis. Mol. Brain Res. 43, 333-337.

Gow A, Gragerov A, Gard A, Colman DR, Lazzarini RA (1997) Conservation of topology, but not conformation, of the proteolipid proteins of the myelin sheath. J. Neurosci. 17, 181-189.

Tosic M, Matthey B, **Gow A**, Lazzarini RA, Matthieu J-M (1997) Intracellular transport of the DM-20 bearing shaking pup (*shp*) mutation and its possible phenotypic consequences. J. Neurosci. Res. 50, 844-852.

Gow A (1997) Redefining the lipophilin family of proteolipid proteins. J. Neurosci. Res. 50, 659-664.

Gow A, Southwood CM, Lazzarini RA (1998) Disrupted proteolipid protein trafficking results in oligodendrocyte apoptosis in animal models of Pelizaeus-Merzbacher disease. J. Cell Biol. 140, 925-934.

Gow A (1999) Myelin oligodendrocyte glycoprotein (MOG). in *Guidebook to the Extracellular Matrix and Adhesion Proteins*. (Kreis T and Vale R, eds) Oxford University Press.

Gow A (1999) Proteolipid proteins (lipophilins). in *Guidebook to the Extracellular Matrix and Adhesion Proteins*. (Kreis T and Vale R, eds) Oxford University Press.

Gow A, Southwood CM, Li JS, Pariali M, Bronstein JM, Riordan GP, Kachar B, Lazzarini RA (1999) CNS Myelin and Sertoli cell tight junction strands are absent in *Osp/Claudin 11*-null mice. Cell 99, 649-659.

Stecca B, Southwood CM, Kelley K, Gragerov A, Friedrich Jr VF, **Gow A** (2000) The evolution of lipophilin genes from invertebrates to tetrapods: DM-20 does not substitute for proteolipid protein in CNS myelin. J. Neurosci. 20, 4002-4010.

Southwood CM, **Gow A** (2001) Functions of OSP/claudin 11-containing parallel tight junctions: implications from the knockout mouse. in *Tight Junctions*. (Anderson JM and Cereijido M, eds) 2nd Edn. CRC Press. pp. 723-745.

Southwood CM, **Gow A** (2001) Insights into the mechanisms of oligodendrocyte apoptosis from hypomyelinated mice. Micros. Res. Tech. 52, 700-708.

Southwood CM, Garbern J, Jiang W, **Gow A** (2002) The unfolded protein response modulates disease severity in Pelizaeus-Merzbacher Disease. Neuron, 36, 585-596.

Gow A (2002) The COS-7 cell in vitro paradigm to study myelin proteolipid protein 1 gene mutations. in: *Methods in Molecular Medicine,* (Potter T, ed), Humana Press, Totowa and New Jersey, pp. 263-275.

Shy M, Hobson G, Boespflug-Tanguy O, Garbern J, Sperle K, Jain M, Li W, **Gow A**, Rodriguez D, Bertini E, Mancias P, Krajewski K, Lewis RA, Kamholz J, members of the ENBDD (2003) Schwann cell expression of PLP1 but not its alternatively spliced isoform DM20 is necessary to prevent demyelinating peripheral neuropathy. Ann Neurol, 53, 354-365.

Yang LV, Heng HH, Southwood CM, **Gow A**, Li L (2003) Alternate promoters and polyadenylation regulate tissue-specific expression of Hemogen isoforms during hematopoisis and spermatogenesis. Dev. Dyn. 228, 606-616.

Gow A and Sharma R (2003) The Unfolded Protein Response in Protein Aggregating Diseases. Neuromolec. Med. 4, 73-94.

Gow A (2004) The *Claudin 11* gene. In *Myelin Biology and Disorders*, (Lazzarini, RA ed.), Elsevier Science, Amsterdam, pp. 565-578.

Gow A (2004) Protein misfolding as a disease determinant. In *Myelin Biology and Disorders*, (Lazzarini, RA ed.), Elsevier Science, Amsterdam, pp 1009-1036.

Gow A, Davies C, Southwood CM, Frolenkov G, Chrustowski M, Ng L, Yamauchi D, Marcus DC, Kachar B (2004) Deafness in *Claudin 11*-null mice reveals the critical contribution of basal cell tight junctions to stria vascularis function. J Neurosci, 24, 7051-7062.

Southwood CM, He C, Garbern J, Kamholz J, Arroyo E, **Gow A** (2004) CNS Myelin Paranodes Require Nkx6-2 Homeoprotein Transcriptional Activity for Normal Structure. J Neurosci, 24, 11215-11225.

Xin M, Yue T, Ma Z, Wu F-F, **Gow A**, Lu QR (2005) Myelinogenesis and axonal recognition by oligodendrocytes in brain are uncoupled in *Olig1*-null mice. J Neurosci, 25, 1354-1365.

Hurst S, Garbern J, Trepanier A, **Gow A** (2006) Quantifying the carrier female phenotype in Pelizaeus-Merzbacher disease. Genet Med, 8, 371-378.

Gow A (2006) Major Components Of Myelin In The Mammalian Central and Peripheral Nervous Systems. In: Neuroimmunology In Clinical Practice, Kalman B and Brannagan, III T, eds, Blackwell, New Jersey, pp. 11-25.

Nunes FD, Lopez LN, Lin HW, Davies C, Azevedo RB, **Gow A**, Kachar B (2006) Distinct sub-domain organization and molecular composition of a tight junction with cell-cell adhesion properties. J Cell Sci., 119, 4819-4827.

Southwood CM, Peppi M, Dryden S, Tainsky MA, **Gow A** (2006) Microtubule deacetylases, SirT2 and HDAC6, in the nervous system. Neurochem Res, 32, 187-195.

Southwood CM, Olson K, Wu C-Y, **Gow A** (2007) Novel alternatively-spliced ER retention signal in the cytoplasmic loop of proteolipid protein 1. J Neurosci Res, 85, 471-478.

Sharma R, **Gow A** (2007) Minimal role for caspase-12 in the unfolded protein response in oligodendrocytes in vivo. J Neurochem, 101, 889-897.

Sharma RN, Jiang H, Zhong L, Tseng J, **Gow A** (2007) Minimal Role For Activating Transcription Factor 3 In The Oligodendrocyte Unfolded Protein Response in vivo, J Neurochem, 102, 1703-1712.

Elkouby-Naor L, Abassi Z, Lagziel A, **Gow A,** Ben-Yosef T (2008) Double gene deletion reveals the lack of cooperation between claudin 11 and claudin 14 tight junction proteins. Cell Tissue Res. 333, 427-438..

Devaux J, **Gow A** (2008) Tight Junctions Potentiate The Insulative Properties Of Small CNS Myelinated Axons. J Cell Biol, 183, 909-921.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Joseph Charles LaManna	Professor
eRA COMMONS USER NAME (credential, e.g., agency login) LAMANNA	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Georgetown University, Washington DC	BS	1971	Biology
Duke University, Durham, NC	PhD	1975	Physiology/ Pharmacology

RESEARCH AND PROFESSIONAL EXPERIENCE

1971-1975 NIMH Pre-doctoral Fellowship
1975 Visiting Investigator, Dept. Neurosurg., Loma Linda Med. Univ., Loma Linda, CA
1975-1977 Research Associate, Dept. Physiol/Pharmacol., Duke Univ. Med. Ctr., Durham, NC
1980 Visiting Investigator, 2nd Dept. Physiol., Semmelweiss Med. Sch., Budapest, Hungary
1977-1981 Assistant Professor, Associate Professor (1981) Depts. Neurol. and Physiol/Biophys., U. Miami Med. Sch., Miami, FL
1993-2008 Chairman, Dept. Anatomy, (Acting Chair 1993-2004), Case Western Reserve University Medical School, Cleveland, OH
1981-pres. Professor, (Associate Professor 1981-1990) Depts. Physiology/Biophysics, Neurology and Neuroscience, Case Western Reserve University Medical School, Cleveland, OH

HONORS AND AWARDS

1975-1977 NIH (NINCDS) NRSA Post-doctoral Fellowship
1977 NIH (NHLBI) Young Investigator Award
1978-1981 NIH (NINCDS) Research Career Development Award
1986-1989 American Heart Association, Brain, Lung and Development Research Study Committee
1987-1990 Veterans Administration Merit Review Board for Neurobiology
1993-1996 NIH Neurology B-1 Study Section
1998-1999 American Heart Association, Cleveland Metro Division, Board of Trustees
2001-03, 06- Journal of Applied Physiology, Editorial Board
2001-2004 Member, American Heart Association (National) Brain 1 Peer Review Committee
2002-2005 Member, Society for Neuroscience Program Committee
2002-pres. Brain Research, Editorial Board
2004-pres. Journal of Cerebral Blood Flow & Metabolism, Editorial Board
2007-2011 Board of Directors, International Society for Cerebral Blood Flow & Metabolism
2007- Board of Directors, Winter Conference on Brain Research
2007-2009 Scientific Program Committee Chairman, International Society for Cerebral Blood Flow & Metabolism
2009 President, International Society on Oxygen Transport to Tissue

SELECTED PUBLICATIONS

LaManna JC, MA Haxhiu, KL Kutina-Nelson, S Pundik, B Erokwu, ER Yeh, WD Lust and NS Cherniack: Decreased energy metabolism in brainstem during central respiratory depression in response to hypoxia. J Appl Physiol 81(4):1772-1777 (1996).

Dore-Duffy, P., Balabanov, R., Beaumont, T., Hritz, M.A., Harik, S.I. and LaManna, J.C., Endothelial activation following prolonged hypobaric hypoxia, Microvasc.Res., 57:75-85 (1999).

Kuo, N.-T., Benhayon, D., Przybylski, R.J., Martin, R.J. and LaManna, J.C., Prolonged hypoxia increases vascular endothelial growth factor mRNA and protein in adult mouse brain, J.Appl.Physiol., 86:260-264 (1999).

Hoxworth, J.M., Xu, K., Zhou, Y., Lust, W.D. and LaManna, J.C., Cerebral metabolic profile, selective neuronal loss, and survival of acute and chronic hyperglycemic rats following cardiac arrest and resuscitation, Brain Res. 821:467-479 (1999).

Kreisman, N.R. and JC LaManna: Rapid and slow swelling during hypoxia in the CA1 region of rat hippocampal slices. J Neurophysiol. 82:320-329 (1999).

Pichiule, P., J.C. Chavez, K. Xu, and J.C. LaManna: Vascular endothelial growth factor upregulation in transient global ischemia induced by cardiac arrest and resuscitation in rat brain. Mol Br Res 74: 83-90 (1999).

Agani, F.H., P. Pichiule, J.C. Chavez, and J.C. LaManna: The role of mitochondria in the regulation of hypoxia-inducible factor-1 expression during hypoxia. J. Biol. Chem. 275:35863-35867 (2000).

Chavez JC, F Agani, P Pichiule, and JC LaManna: Expression of hypoxia inducible factor 1 alpha in the brain of rats during chronic hypoxia. J. Appl. Physiol. 89:1937-1942 (2000).

Agani FH, JC Chavez, P Pichiule, M Puchowicz and JC LaManna: The role of nitric oxide in the regulation of HIF-1a expression during hypoxia. Am. J. Physiol.:Cell Physiology 283:C178-C186 (2002).

Pichiule P and JC LaManna: Angiopoietin-2 and rat brain capillary remodeling during adaptation and deadaptation to prolonged mild hypoxia. J. Appl. Physiol. 93:1131-1139 (2002).

Agani F, Pichiule P, Chavez JC, LaManna J. Inhibitors of mitochondrial complex I attenuate the accumulation of hypoxia-inducible factor-1 during hypoxia in Hep3B cells. Comp Biochem and Physiol Part A 132, 107-109 (2002).

Chavez JC and JC LaManna: Activation of hypoxia inducible factor-1 in the rat cerebral cortex after transient global ischemia: potential role of insulin like growth factor-1. J. Neurosci. 22(20):8922-8931 (2002).

Pichiule P, JC Chavez, and JC LaManna: Hypoxic regulation of angiopoietin-2 expression in endothelial cells. *J.Biol.Chem.* 279 (13):12171-12180 (2004).

Puchowicz MA, K Xu, D Magness, C Miller, WD Lust, TS Kern, and JC LaManna: Comparison of glucose influx and blood flow in retina and brain of diabetic rats. *J.Cereb.Blood Flow Metab.* **24**:449-457 (2004).

LaManna JC, JC Chavez, and P Pichiule: Structural and functional adaptation to hypoxia in the rat brain. *J.Exp.Biol.* 207:3163-3169 (2004).

Drew KL, MB Harris, JC LaManna, MA Smith, XW Zhu, and YL Ma: Hypoxia tolerance in Mammalian heterotherms. *J.Exp.Biol.* 207:3155-3162 (2004).

Xu K, MA Puchowicz, and JC LaManna: Renormalization of regional brain blood flow during prolonged mild hypoxic exposure in rats. *Brain Res.* 1027:188-191 (2004).

Ward NL and JC LaManna: The neurovascular unit and its growth factors: coordinated response in the vascular and nervous systems. *Neurol.Res.* 26:870-883 (2004).

Aminova LR, JC Chavez, J Lee, H Rye, A Kung, JC LaManna, and RR Ratan: Pro-survival and pro-death effects of HIF-1α stabilization in a murine hippocampal cell line. *J. Biol. Chem.* 280:3996-4003 (2005).

Zhu X, Smith MA, Perry G, Wang Y, Ross AP, Zhao HW, LaManna JC, Drew KL. MAPKs are differentially modulated in arctic ground squirrels during hibernation. J Neurosci Res. 80(6):862-868 (2005).

Siddiq A, Ayoub IA, Chavez JC, Aminova L, Shah S, LaManna JC, Patton SM, Connor JR, Cherny RA, Volitakis I, Bush A, Langsetmo I, Seeley T, Gunzler V, Ratan RR. HIF prolyl 4-hydroxylase inhibition: A target for neuroprotection in the central nervous system. J Biol Chem. 280 (50), 41732-41743 (2005).

Ma YL, Zhu X, Rivera PM, Toien O, Barnes BM, LaManna JC, Smith MA, Drew KL., Absence of cellular stress in brain after hypoxia induced by arousal from hibernation in Arctic ground squirrels. Am J Physiol Regul Integr Comp Physiol. 289(5):R1297-306 (2005).

Xu K, MA Puchowicz, WD Lust, and JC LaManna: Adenosine treatment delays postischemic hippocampal CA1 loss after cardiac arrest and resuscitation in rats. Br Res 1071:208-217 (2006).

Xu K and JC LaManna: Chronic hypoxia and the cerebral circulation. J Appl Physiol 100:725-730 (2006).

Fisher EM, RP Steiner, and JC LaManna: Intracellular pH in gastric and rectal tissue post cardiac arrest. *Adv.Exp.Med.Biol.* **578**:11-16 (2006).

Kanaan A, R Farahani, RM Douglas, JC LaManna, and GG Haddad: Effect of chronic continuous or intermittent hypoxia and reoxygenation on cerebral capillary density and myelination. *Am J Physiol Regul.Integr.Comp Physiol* **290**:R1105-R1114 (2006).

Ward NL, Moore E, Noon K, Spassil N, Keenan E, Ivanco TL, LaManna JC. Cerebral angiogenic factors, angiogenesis, and physiological response to chronic hypoxia differ among four commonly used mouse strains. J Appl Physiol. **102**(5):1927-35 (2007).

Occhipinti R, Puchowicz, MA, LaManna JC, Somersalo E, and Calvetti D: Statistical analysis of metabolic pathways of brain metabolism at steady state. *Ann Biomed Eng* ***35***(6):886-902 (2007).

Puchowicz M, Xu K, Sun X, Ivy A, Emancipator D, and JC LaManna: Diet-induced ketosis increases capillary density without altered blood flow in rat brain. *AJP: Endocrinology and Metabolism 292:E1607-E1615 (2007).*

Ndubuizu O and LaManna JC: Brain tissue oxygen concentration measurements. *Antioxidants & Redox Signaling* 9 (8), 1207-1219 (2007).

Dore-Duffy PD and JC LaManna: Physiological angiodynamics in the brain. *Antioxidants & Redox Signaling* 9 (9): 1363-1371 (2007).

LaManna JC: Hypoxia in the central nervous system. *Essays in Biochemistry* **43**:139-152 (2007).

Bishop GM, MA Smith, JC LaManna, AC Wilson, G Perry, and CS Atwood: Iron homeostasis is maintained in the brain, but not the liver, following mild hypoxia. *Redox.Rep.* **12**:257-266 (2007).

LaManna JC: In situ measurements of brain tissue hemoglobin saturation and blood volume by reflectance spectrophotometry in the visible spectrum. *J Biomed.Opt.* **12**:062103(2007).

Ratan RR, A Siddiq, N Smirnova, K Karpisheva, R Haskew-Layton, S McConoughey, B Langley, A Estevez, PT Huerta, B Volpe, S Roy, CK Sen, I Gazaryan, S Cho, M Fink, and J LaManna: Harnessing hypoxic adaptation to prevent, treat, and repair stroke. *J Mol.Med.* **85**:1331-1338 (2007).

Milner R, S Hung, B Erokwu, P Dore-Duffy, JC LaManna and GJ del Zoppo: Increased expression of fibronectin and the alpha-5-beta-1 integrin in angiogenic cerebral blood vessels of mice subject to hypobaric hypoxia. Mol. Cell. Neurosci. 38:43-52 (2008).

Puchowicz MA, J Zechel, J Valerio, D Emancipator, K Xu, S Pundik, JC LaManna and WD Lust: Neuroprotection in Diet Induced Ketotic Rat Brain Following Focal Ischemia. *J Cereb Blood Flow Metab* 28:1907-1916 (2008).

Xu, K and JC LaManna: The loss of hypoxic ventilatory responses following resuscitation after cardiac arrest in rats is associated with failure of long-term survival. *Br Res* 1258, 59-64 (2009).

Ndubuizu OI, JC Chavez, and JC LaManna: Increased Prolyl 4-Hydroxylase (PHD) Expression and Differential Regulation of Hypoxia Inducible Factors (HIFs) in the Aged Rat Brain. *AJP Reg* (in press).

RESEARCH PROJECTS ONGOING, PENDING OR COMPLETED DURING THE LAST 3 YEARS:

Active:

"Brain vascular and metabolic adaptations to hypoxia", Principal Investigator: Joseph C. LaManna; USPHS NIH; Type: R01 NS 38632, Period: 7-15-05–6-30-10

We propose to examine the structural aspects of the rat brain adaptation to prolonged continuous and intermittent hypoxia.

"Angiogenic Response to Hypoxia and Ketosis in Rat Brain", PI: JC LaManna; Agency: USPHS NIH; Type: 1R01 HL092933-01A1; Project Period: 4-1-09 – 3-31-2014

We propose to investigate whether ketosis induces angiogenesis in aged brain and to investigate if ketosis induces HIF-1 alpha in aged rat brain and improves adaptation to mild hypoxia.

"**Energy Balance During Ketosis in Rat Brain"**, PI: JC LaManna; Agency: USPHS NIH NHLBI; Type: R21 NS062048; Project Period: 5-1-09 – 4-30-11

The goal of this project is to investigate the effects of ketosis on brain metabolism of glucose (CMRglu) using image (PET) analysis in ketotic rat and to determine if there is improved outcome following cardiac arrest and resuscitation.

"Nursing Inquiry: Generation of Gut CO_2 Under Dysoxic Conditions in rats", PI: Elaine M. Fisher; Sponsor: Joseph C. LaManna; NIH NINR Type: K01 NR009787-01; Period: 3/1/06 – 2/28/09

"Role of the PVN in Chronic Intermittent Hypoxia-Induced Cardiorespiratory Changes", PI: P Kc; Co-Mentors: JC LaManna and RJ Martin, USPHS NIH 1K99/R00 HL087620; Period: 1/1/08 -12/31/12

"An Angiogenic Role for the alpha-5-beta-1 and alpha-v-beta-3 Integrins During Cerebral Ischemia", Subcontract Project PI: JC LaManna, Agency: NIH/Scripps Research Institute; Type: R01 NS060770; Period: 715/08 – 6/30/09

The goal of this project is to examine the notion that cerebral ischemia induces endothelial cell expression of the $\alpha5\beta1$ and $\alpha v\beta3$ integrins, and that fibronectin promotes new blood vessel formation in the brain via these integrins.

"Collaborative MS Research Center Award", PI: PD Duffy, Outside Collaborator: JC LaManna, National Multiple Sclerosis Society, Period: 4-1-07 – 3-31-12

"Mechanisms of Neonatal Erythropoietin Neuroprotection", PI: S Robinson, Consultant: JC LaManna Agency: USPHS NIH R01 NS060765-01A1

"The Jeannette M. and Joseph S. Silber Research Fund for the Study of Brain Sciences", PI: JC LaManna (Private Fund); Period: 8/2008 –
This is a research fund which was established to improve the outcome of stroke patients.

Training Grant Participation for both pre and postdoctoral fellows (no salary):
"Cleve. Training Prog. in Cardiovascular Research", G Dubyak, T32HL07653, 4-1-04--3-31-09
"Sleep Medicine: Neurobiology and Epidemiology", KP Strohl, T32NS07913; 7-1-05--6-30-10
"Heart-Lung Physiology: Molecular-Systemic Integration", WP Schilling, T32HL07887; 7-1-04 --6-30-09
"Medical Scientist Training Program", C Harding, T32 GM007250; 7-1-95 – 6-30-09

Pending:

"Heat-shock protein co-inducer for inducing rapid altitude & hypoxia acclimatization", PI: Ibolja Cernak (Johns Hopkins APL); Subcontract PI: JC LaManna; Agency: DARPA (Defense Advanced Research Projects Agency, BAA0-09-09; Proposed Project Period: 3-1-09 – 3-30-11

"Role of COX-2 – Ang-2 signaling in vascular remodeling in aging mouse brain", PI: JC LaManna, PhD Student: Girriso Benderro; Agency: Alzheimer's Association, Appl. #2009ALZ00IIRG000039698; Proposed Project Period: 7-1-09 – 6-30-12

"Role of Angiopoietin-2 (Ang-2) in vascular remodeling during hypoxia in aging mouse brain", PI: Girriso Benderro (PhD Student), Sponsor: JC LaManna; Agency: American Heart Association (Great Rivers Affiliate); Type: Predoctoral Fellowship, Appl. #09PRE2250387; Proposed Project Period: 7-1-09 – 6-30-11

"Refinement of lead compounds to modulate water permeability through AQP4", PI: WF Boron (Aeromics); Type: Phase II SBIR; Consultant: JC LaManna

"Predictive models of cerebral metabolic changes leading to neuroprotection by dietary ketogenesis in the aging brain", PI: D Calvetti, Co-PI: JC LaManna, Agency: NIH; Type: RC1 Grant; Proposed Project Period: 9-10-09 – 8-31-11

"Recovery Act Administrative Supplement for Students/Science Educators, under R01 HL092933 – Angiogenic Response to Hypoxia and Ketosis in Rat Brain"; PI: JC LaManna; Agency: NIH NHLBI; Type: Admin. Supplement, NOT-OD-09-060; Proposed Project Period: 7-1--09 – 6-30-11 (to hire 2 undergraduate summer students, one in 2009, the other in 2010)

"Recovery Act Administrative Supplement, under R01 HL092933 – Angiogenic Response to Hypoxia and Ketosis in Rat Brain"; PI: JC LaManna; Agency: NIH NHLBI; Type: Admin. Supplement, NOT-OD-09-056; Proposed Project Period: 7-1--09 – 6-30-11 (to hire a recently graduated Master's BME student into a predoctoral position)

"NINDS ARRA Administrative Supplement, under R01 NS38632 – Brain Vascular and Metabolic Response to Hypoxia", PI: JC LaManna; Agency: NIH NINDS; Type: Admin. Supplement, NOT-OD-09-056; Proposed Project Period: 6-1-09 – 5-31-10 (to hire an animal person for breeding mice)

Completed:

"Pilot Study: Improving Warfighters' Sustainment and Performance in Extreme Environmental Conditions", PI: Ibolja Cernak (Johns Hopkins APL); Subcontract PI: JC LaManna, Project, "Simulated-altitude adaptation study"; Agency: DARPA (Defense Advanced Research Projects Agency) US Army ECBC, Cont. # W911-NF-07-C-0053; APL Cont. # 929408; Project Period: 7/1/07 – 2/14/08 -- We tested a drug to find out if it supports adaptation to hypoxia.

"Treatment Strategies in a Rat Model of Cardiac Arrest", Principal Investigator: Joseph C. LaManna USPHS NIH; Type: 1R01 NS46074, Period: 9-30-02 – 6-30-07 -- We proposed to investigate new treatment strategies aimed at using alternate energy substrates such as, pyruvate and ketones in combination with antioxidant type drugs, such as, melatonin, N-t-Butyl-α-Phenyl-nitrone, adenosine and methylisobutyl amiloride, as therapies for improving recovery from cardiac arrest.

"Center for Modeling Integrated Metabolic Systems (MIMS)", PI: G.M. Saidel; Project: J.C. LaManna USPHS NIH; Type: P50 GM066309-01; Period: 7-10-02 – 6-30-07 -- This was an application to establish and support a center for modeling integrated metabolic systems.

"The Kenneth Haas Medical Care Trust", Principal Investigators: Joseph C. LaManna and Jose Suarez Type: Private Fund; Period 2001 -- 2007 -- This was a translational research project in which researchers and clinicians worked closely to produce the best possible outcome to cardiac arrest patients.

OMB Number: 4040-0001
Expiration Date: 06/30/2011

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** 0019622240000

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Wayne State University

Delete Entry *** Start Date:** 04/01/2010 *** End Date:** 03/31/2011 **Budget Period** 1

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Paula		Dore-Duffy	Ph.D.	PD/PI	183,419.00	3.00			45,855.00	11,693.00	57,548.00
2.	Dr.	Jose		Rafols	PhD	Co-PI	174,791.00	1.20			17,479.00	4,457.00	21,936.00
3.	Dr.	Christian		Kreipke	PhD	Co-PI	44,887.00	1.20			4,489.00	1,145.00	5,634.00
4.	Dr.	Alexander		Gow	PhD	Co-PI	115,195.00	0.60			5,760.00	1,469.00	7,229.00
5.													
6.													
7.													
8.													

9. **Total Funds requested for all Senior Key Persons in the attached file**

Total Senior/Key Person 92,347.00

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Vladimir Katyshev, M.D., Research Assistant	12.00			29,994.00	7,648.00	37,642.00
1	Alexander Tapper, B.S., Research Assistant	6.00			13,000.00	3,315.00	16,315.00
1	To be Named (Ph.D, Post-Doctoral)	12.00			35,000.00	8,925.00	43,925.00
3	**Total Number Other Personnel**					**Total Other Personnel**	97,882.00

Total Salary, Wages and Fringe Benefits (A+B) 190,229.00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

* ORGANIZATIONAL DUNS: [redacted]

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: [redacted]

Delete Entry | * Start Date: [redacted] * End Date: [redacted] Budget Period 1

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

	Equipment item	* Funds Requested ($)
1.	Normobaric Hypoxic Chambers, (2)	16,500.00
2.	OxyCycler for chambers (1)	45,650.00
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.	**Total funds requested for all equipment listed in the attached file**	
	Total Equipment	62,150.00

Additional Equipment: | Add Attachment | Delete Attachment | View Attachment

D. Travel

		Funds Requested ($)
1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	4,000.00
2.	Foreign Travel Costs	
	Total Travel Cost	4,000.00

E. Participant/Trainee Support Costs

		Funds Requested ($)
1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	
	Number of Participants/Trainees	**Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 1

Next Period

* ORGANIZATIONAL DUNS: [redacted]

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: [redacted]

Delete Entry | Start Date: 04/01/2010 | * End Date: 03/31/2011 | Budget Period 1

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	98,500.00
2. Publication Costs	2,500.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Image Analyses (MRI)	7,500.00
9. Laboratory Equipment Maintenance	4,500.00
10. Software($2500) / Shipping Charges ($500)	3,000.00
Total Other Direct Costs	116,000.00

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	372,379.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1. Modified Total Direct Costs	52.00	310,229.00	161,319.00
2.			
3.			
4.			
Total Indirect Costs			161,319.00

Cognizant Federal Agency US Department of Education-Richard Dowd, 312-886-6503
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	533,698.00

J. Fee	Funds Requested ($)

K. * Budget Justification 1256-BudgJust NIH June 2009.pdf (Only attach one file.)

Add Attachment | Delete Attachment | View Attachment

Tracking Number:GRANT10340434 Funding Opportunity Number:PA-08-015 Received Date:2009-06-05T10:42:03-04:00

OMB Number: 4040-0001
Expiration Date: 06/30/2011

Previous Period

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

*** ORGANIZATIONAL DUNS:** 0019622240000

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Wayne State University

Delete Entry *** Start Date:** 04/01/2011 *** End Date:** 03/31/2012 **Budget Period** 2

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Paula		Dore-Duffy	Ph.D.	PD/PI	188,922.00	3.00			47,230.00	12,044.00	59,274.00
2.	Dr.	Jose		Rafols	PhD	Co-PI	180,035.00	1.20			18,003.00	4,591.00	22,594.00
3.	Dr.	Christian		Kreipke	PhD	Co-PI	46,234.00	1.20			4,623.00	1,179.00	5,802.00
4.	Dr.	Alexander		Gow	PhD	Co-PI	118,651.00	0.60			5,933.00	1,514.00	7,447.00
5.													
6.													
7.													
8.													

9. **Total Funds requested for all Senior Key Persons in the attached file**

Total Senior/Key Person 95,117.00

Additional Senior Key Persons: Add Attachment | Delete Attachment | View Attachment

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Vladimir Katyshev, M.D., Research Assistant	12.00			30,893.00	7,878.00	38,771.00
1	Alexander Tapper, B.S., Research Assistant	6.00			13,390.00	3,415.00	16,805.00
1	To be named (Ph.D, Post-Doctoral)	12.00			36,050.00	9,193.00	45,243.00
3	**Total Number Other Personnel**					**Total Other Personnel**	100,819.00

Total Salary, Wages and Fringe Benefits (A+B) 195,936.00

Tracking Number:GRANT10340434 Funding Opportunity Number:PA-08-015 Received Date:2009-06-05T10:42:03-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

* ORGANIZATIONAL DUNS:

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization:

Delete Entry | * Start Date: | * End Date: | Budget Period 2

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

	Equipment item	* Funds Requested ($)
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.	**Total funds requested for all equipment listed in the attached file**	
	Total Equipment	

Additional Equipment: Add Attachment | Delete Attachment | View Attachment

D. Travel

		Funds Requested ($)
1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	4,120.00
2.	Foreign Travel Costs	
	Total Travel Cost	4,120.00

E. Participant/Trainee Support Costs

		Funds Requested ($)
1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	
	Number of Participants/Trainees	**Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 2

Next Period

* ORGANIZATIONAL DUNS: [redacted]

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Wayne State University

Delete Entry | Start Date: 04/01/2011 | * End Date: 03/31/2012 | Budget Period 2

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	101,455.00
2. Publication Costs	2,575.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Image Analyses (MRI)	7,725.00
9. Laboratory Equipment maintenance	4,635.00
10. Software ($2575) / Shipping charges($515)	3,090.00
Total Other Direct Costs	119,480.00

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	319,536.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1. Modified Total Direct Costs	52.00	319,536.00	166,159.00
2.			
3.			
4.			
Total Indirect Costs			166,159.00

Cognizant Federal Agency US Department of Education, Richard Dowd, 312-886-6503
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	485,695.00

J. Fee	Funds Requested ($)

K. * Budget Justification 1256-BudgJust NIH June 2009.pdf | Add Attachment | Delete Attachment | View Attachment
(Only attach one file.)

OMB Number: 4040-0001
Expiration Date: 06/30/2011

Previous Period

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

*** ORGANIZATIONAL DUNS:** 0019622240000

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Wayne State University

Delete Entry *** Start Date:** 04/01/2012 *** End Date:** 03/31/2013 **Budget Period** 3

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Paula		Dore-Duffy	Ph.D.	PD/PI	194,590.00	3.00			48,647.00	12,405.00	61,052.00
2.	Dr.	Jose		Rafols	PhD	Co-PI	185,436.00	1.20			18,544.00	4,729.00	23,273.00
3.	Dr.	Christian		Kreipke	PhD	Co-PI	47,621.00	1.20			4,762.00	1,214.00	5,976.00
4.	Dr.	Alexander		Gow	PhD	Co-PI	122,211.00	0.60			6,111.00	1,558.00	7,669.00
5.													
6.													
7.													
8.													
9.	**Total Funds requested for all Senior Key Persons in the attached file**												

Total Senior/Key Person 97,970.00

Additional Senior Key Persons: Add Attachment | Delete Attachment | View Attachment

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Vladimir Katyshev, M.D., Research Assistant	12.00			31,820.00	8,114.00	39,934.00
1	Alexander Tapper, B.S, Research Assistant	6.00			13,792.00	3,517.00	17,309.00
1	To be named (Ph.D, Post-Doctoral)	12.00			37,132.00	9,469.00	46,601.00
3	**Total Number Other Personnel**					**Total Other Personnel**	103,844.00

Total Salary, Wages and Fringe Benefits (A+B) 201,814.00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

* ORGANIZATIONAL DUNS: [illegible]

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: [illegible]

Delete Entry * Start Date: 04/01/2012 * End Date: 03/31/2013 Budget Period 3

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

	Equipment item	* Funds Requested ($)
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.	**Total funds requested for all equipment listed in the attached file**	
	Total Equipment	

Additional Equipment: Add Attachment Delete Attachment View Attachment

D. Travel

		Funds Requested ($)
1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	4,244.00
2.	Foreign Travel Costs	
	Total Travel Cost	4,244.00

E. Participant/Trainee Support Costs

		Funds Requested ($)
1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	
	Number of Participants/Trainees	**Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 3

Next Period

* ORGANIZATIONAL DUNS: 381962220000

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Wayne State University

Delete Entry | Start Date: 04/01/2012 | * End Date: 03/31/2013 | Budget Period 3

F. Other Direct Costs		Funds Requested ($)
1.	Materials and Supplies	104,499.00
2.	Publication Costs	2,652.00
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Image Analyses (MRI)	7,957.00
9.	Laboratory Equipment maintenance	4,774.00
10.	Software ($2652) / Shipping charges($530)	3,182.00
	Total Other Direct Costs	123,064.00

G. Direct Costs		Funds Requested ($)
	Total Direct Costs (A thru F)	329,122.00

H. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	Modified Total Direct Costs	52.00	329,122.00	171,143.00
2.				
3.				
4.				
	Total Indirect Costs			171,143.00

Cognizant Federal Agency US Department of Education, Richard Dowd, 312-886-6503
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs		Funds Requested ($)
	Total Direct and Indirect Institutional Costs (G + H)	500,265.00

J. Fee	Funds Requested ($)

K. * Budget Justification 1256-BudgJust_NIH_June_2009.pdf | Add Attachment | Delete Attachment | View Attachment
(Only attach one file.)

OMB Number: 4040-0001
Expiration Date: 06/30/2011

Previous Period

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

* ORGANIZATIONAL DUNS: 0019622240000

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Wayne State University

Delete Entry * Start Date: 04/01/2013 * End Date: 03/31/2014 Budget Period 4

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Paula		Dore-Duffy	Ph.D.	PD/PI	200,427.00	3.00			50,107.00	12,777.00	62,884.00
2.	Dr.	Jose		Rafols	PhD	Co-PI	190,999.00	1.20			19,100.00	4,871.00	23,971.00
3.	Dr.	Christian		Kreipke	PhD	Co-PI	49,050.00	1.20			4,905.00	1,251.00	6,156.00
4.	Dr.	Alexander		Gow	PhD	Co-PI	125,877.00	0.60			6,294.00	1,605.00	7,899.00
5.													
6.													
7.													
8.													
9.	Total Funds requested for all Senior Key Persons in the attached file												

Total Senior/Key Person 100,910.00

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Vladimir Katyshev, M.D., Research Assistant	12.00			32,775.00	8,358.00	41,133.00
1	Alexander Tapper, B.S., Research Assistant	6.00			14,205.00	3,622.00	17,827.00
1	To be named (Ph.D, Post-Doctoral)	12.00			38,245.00	9,752.00	47,997.00
3	Total Number Other Personnel					Total Other Personnel	106,957.00

Total Salary, Wages and Fringe Benefits (A+B) 207,867.00

Tracking Number:GRANT10340434
Funding Opportunity Number:PA-08-015 Received Date:2009-06-05T10:42:03-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

* ORGANIZATIONAL DUNS: [redacted]

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: [redacted]

Delete Entry * Start Date: [redacted] * End Date: [redacted] Budget Period 4

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

	Equipment item	* Funds Requested ($)
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.	**Total funds requested for all equipment listed in the attached file**	
	Total Equipment	

Additional Equipment: Add Attachment Delete Attachment View Attachment

D. Travel

		Funds Requested ($)
1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	4,371.00
2.	Foreign Travel Costs	
	Total Travel Cost	4,371.00

E. Participant/Trainee Support Costs

		Funds Requested ($)
1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	
	Number of Participants/Trainees	**Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 4

Next Period

* ORGANIZATIONAL DUNS: 001960224[illegible]

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Wayne State University

Delete Entry | * Start Date: 04/01/2013 | * End Date: 03/31/2014 | Budget Period 4

F. Other Direct Costs		Funds Requested ($)
1.	Materials and Supplies	107,634.00
2.	Publication Costs	2,732.00
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Image Analyses (MRI)	8,195.00
9.	Laboratory Equipment Maintenance	4,917.00
10.	Software ($2732) / Shipping charges($546)	3,278.00
	Total Other Direct Costs	126,756.00

G. Direct Costs		Funds Requested ($)
	Total Direct Costs (A thru F)	338,994.00

H. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	Modified Total Direct Costs	52.00	338,994.00	176,277.00
2.				
3.				
4.				
	Total Indirect Costs			176,277.00

Cognizant Federal Agency US Department of Education, Richard Dowd, 312-886-6503
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs		Funds Requested ($)
	Total Direct and Indirect Institutional Costs (G + H)	515,271.00

J. Fee	Funds Requested ($)

K. * Budget Justification 1256-BudgJust NIH June 2009.pdf | Add Attachment | Delete Attachment | View Attachment
(Only attach one file.)

OMB Number: 4040-0001
Expiration Date: 06/30/2011

Previous Period

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

*** ORGANIZATIONAL DUNS:** 0019622240000

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Wayne State University

Delete Entry *** Start Date:** 04/01/2014 *** End Date:** 03/31/2015 **Budget Period** 5

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Paula		Dore-Duffy	Ph.D.	PD/PI	206,440.00	3.00			51,610.00	13,161.00	64,771.00
2.	Dr.	Jose		Rafols	PhD	Co-PI	196,729.00	1.20			19,673.00	5,017.00	24,690.00
3.	Dr.	Christian		Kreipke	PhD	Co-PI	50,521.00	1.20			5,052.00	1,288.00	6,340.00
4.	Dr.	Alexander		Gow	PhD	Co-PI	129,653.00	0.60			6,483.00	1,653.00	8,136.00
5.													
6.													
7.													
8.													
9.	**Total Funds requested for all Senior Key Persons in the attached file**												

Total Senior/Key Person 103,937.00

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Vladimir Katyshev, M.D., Research Assistant	12.00			33,758.00	8,608.00	42,366.00
1	Alexander Tapper, B.S., Research Assistant	6.00			14,632.00	3,731.00	18,363.00
1	To be named (Ph.D, Post-Doctoral)	12.00			39,393.00	10,045.00	49,438.00
3	**Total Number Other Personnel**					**Total Other Personnel**	110,167.00

Total Salary, Wages and Fringe Benefits (A+B) 214,104.00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

* ORGANIZATIONAL DUNS: [redacted]

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Wayne State University

Delete Entry | * Start Date: 07/01/2014 | * End Date: 06/30/2015 | Budget Period 5

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

	Equipment item	* Funds Requested ($)
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.	**Total funds requested for all equipment listed in the attached file**	
	Total Equipment	

Additional Equipment: | Add Attachment | Delete Attachment | View Attachment

D. Travel

		Funds Requested ($)
1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	4,502.00
2.	Foreign Travel Costs	
	Total Travel Cost	4,502.00

E. Participant/Trainee Support Costs

		Funds Requested ($)
1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	
	Number of Participants/Trainees	**Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 5

* ORGANIZATIONAL DUNS: 831962220000

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Wayne State University

Delete Entry | Start Date: 08/01/2014 | * End Date: 03/31/2015 | Budget Period 5

F. Other Direct Costs		Funds Requested ($)
1.	Materials and Supplies	110,863.00
2.	Publication Costs	2,814.00
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Image analyses (MRI)	8,440.00
9.	Laboratory Equipment Maintenance	5,065.00
10.	Software ($2814) / Shipping ($562)	3,376.00
	Total Other Direct Costs	130,558.00

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	349,164.00

H. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	Modified Total Direct Costs	52.00	349,164.00	181,565.00
2.				
3.				
4.				
	Total Indirect Costs			181,565.00

Cognizant Federal Agency US Department of Education, Richard Dowd, 312-886-6503
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	530,729.00

J. Fee	Funds Requested ($)

K. * Budget Justification 1256-BudgJust NIH June 2009.pdf (Only attach one file.) | Add Attachment | Delete Attachment | View Attachment

Budget Justification

Personnel

Paula Dore-Duffy, Ph.D. – Principal Investigator (3.0 calendar months of effort) is a Professor in the Department of Neurology at Wayne State University School of Medicine and also holds an associate appointment in the Department of Immunology and Microbiology. Dr. Dore-Duffy is Chief of the Division of Neuroimmunology and Director of Research for the Wayne State University Multiple Sclerosis Clinical Research Center. Her translational research interests include the roles of the blood brain barrier (BBB) in neurodegenerative diseases, such as multiple sclerosis, traumatic brain injury (TBI), and stroke. Her basic science research interests include cell-cell interactions in the BBB and the role of CNS capillary pericyte in angiodynamics. Dr. Dore-Duffy has been a member of the review committees for the MS Society, VAH, and the National Institutes of Health, the Department of Defense, and the American Heart Association. She is also a new member of the American Heart Association. Dr. Dore-Duffy is the Deputy Editor for the Journal of Neurological Sciences and is on the Editorial Board for three other journals, including Microvascular Research. She is a regular reviewer for a number of vascular biological, neurological, and immunological journals.

Dr. Dore-Duffy's role in this proposal will be to direct key personnel and to design experiments to be performed to meet the stated objectives. Dr. Dore-Duffy is requesting partial salary support at the University-mandated percentage of 25%. She will be responsible for the overall direction and management and administration of this project.

Jose Rafols, Ph.D. – Co-Principal Investigator (1.2 calendar months of effort); He is a Professor in the Department of Anatomy and Cell Biology. Dr Rafols will oversee the pathological and histological characterization of angiodynamics. He is an expert in neuroanatomy and has extensive experience at assessing and diagnosing TBI and developing therapies for the treatment of TBI in animal models. The mouse model of TBI is housed in his laboratory.

Christian Kreipke, Ph.D. – Co- Principal Investigator (1.2 calendar months of effort); Dr. Kreipke is an Assistant Professor in the Department of Anatomy and Cell Biology. He will be assisting Dr. Rafols in performing and overseeing the modified Marmarou Model in mice. Additionally, he will train and oversee the performance of research assistants and associates as they conduct experiments to determine the effect of normobaric hypoxia on the neuropathologic/cognitive outcomes following TBI.

Alexander Gow, Ph.D – Co-Investigator (0.6 calendar months of effort); Dr. Alex Gow is Associate Professor of Pediatrics and has an appointment in the Departments of Molecular Medicine and Neurology. Dr. Gow's research interests are in stress responses that are involved in the unfolded protein response in demyelination. He has an ongoing collaboration with Dr. Dore-Duffy that studies the role of UPR in VEGF and other HIF-1α directed mechanisms in EAE models. Dr. Gow is a developmental molecular cellular neurobiologist with 19 years experience in designing, generating and analyzing transgenic and knockout mice at the levels of Southern, northern, western blotting, immunocytochemistry, confocal and electron microscopy. He has 11 years experience analyzing mutant phenotypes at the levels evoked potentials. His laboratory has generated the transgenic mice use in the studies. Dr Gow will oversee all of the experiments associated with the transgenic mice in this project and manuscript writing.

Vladimir Katyshev, M.D. – Research Assistant (12 calendar months of effort); Dr. Katyshev has been involved in the BBB research for the past six years. He has developed and currently

has experience in all the molecular biological techniques proposed in this application. Dr Katyshev is also trained in tissue culture techniques, Western blots, and capillary isolation. He will assist Dr Dore-Duffy in the direction of the laboratory personnel and data analysis. The request is made for 100% of his salary.

Alexander Tapper, B.S. – Research Assistant (6 calendar months of effort); Alex is a research assistant in the laboratory who has been trained in the isolation of CNS capillaries and in the subculture and propagation of pericytes. He has also been trained to perform immunological techniques, immunocytochemistry, and image analysis, using MetaMorph and ImageJ software. His role in the study will be to dissect all animals and harvest tissue for sectioning, immunocytochemistry, and for culture. Funds for the remainder of his salary will be provided by research funding available to Dr. Dore-Duffy through currently funded projects related to this proposal.

To Be Named, Ph.D. – (12 calendar months of effort); this individual will be a postdoctoral fellow with training in molecular and cell biology and experience with transgenic mice. S/he will help manage the *EMH-CreER^{T2}* colony, genotype the mice, participate in TBI experiments outlined in Specific Aim #3, and perform the histochemistry and immunofluorescence experiments.

Joseph LaManna, Ph.D. – Consultant; Professor of Physiology and Biophysics with a secondary appointment in Neurology and Neuroscience at Case Western Reserve University. He has been active in cerebrovascular and metabolic research full time for more than 25 years. Dr LaManna is a leading expert in physiological angiogenesis. He has been primarily interested in the integrated systems physiology of the coupled interactions between the brain microvasculature and the metabolic demands set by neuronal and glial activity. Dr. LaManna will consult on experiments involving normobaric hypoxia and HIF-1α.

Equipment

Our laboratories are well equipped to conduct the proposed experiments. However, as we are funded to conduct experiments in EAE animals that use the chambers, the two existing chambers are consistently full and in use. We feel that two more chambers are needed. We have a quote from Biospherix, a leading manufacturer of hypoxic chambers for animal and cell culture work in the amount of $62,150 (this quote was generated May 20, 2009). This price includes hardware for cycling and controlling oxygen levels, a laptop PC to log all data and allow for remote access, the proprietary software, training/SOPs, and periodic upgrades/service. The OxyCycler is requested to adjust for intermittent oxygen levels. We have requested an amount equal to the price increase for this company for past several years to compensate for any variance in the price of these items.

Supplies

Molecular reagents and general laboratory disposables – We will perform various immune-based assays, including ELISAs, Western blots, dual immunolabeled tissue stains, FACs analysis, and molecular biological techniques. The majority of our supplies will include antibodies, molecular reagents, hematocrit kits, glass slides and coverslips, tissue culture equipment, random-priming kits, digoxigenin RNA labeling/detection kits for in situ hybridization, tail DNA purification kits, restriction enzymes, PCR kits, genotyping supplies, Taq polymerase,

and additional supplies for protein isolations and the hypoxic chamber (including oxygen and nitrogen gas, and replacement tubing). Additionally, we are requesting general laboratory chemicals/reagents/plasticware/glassware/disposables in our budget - dishes for bacterial culture; media; sterile pipettes; 15 and 50 ml falcon tubes; 15 ml Falcon polypropylene tubes for tissue storage, transformations, tissue homogenization; Eppendorf tubes for enzyme reactions, tail DNA purification, PCR; Gilson pipette tips; large volume pipettes, Pasteur pipettes, beakers, conical flasks for bacterial cultures; acids/alkali/salts for Southern and northern blotting; cesium chloride for RNA preparations; SDS polyacrylamide gel electrophoresis reagents, buffers, organic solvents for tail DNA extraction and precipitation.

Radioisotopes – ^{32}P trinucleotide phosphates to synthesize random-primed probes for northern and Southern blot analyses and cell labeling experiments.

Animal dissection/tissue harvesting – Sterile scalpels; disposable blades; scissors and yearly maintenance (sharpening costs); paraffin and OCT (media for embedding tissues); chemicals and solvents for processing paraffin and cryostat samples; microtome blades; PAP pens; glass microscope slides; nail polish or other sealing resin for glass slides; fixatives.

Travel

Funds for traveling to conferences and scientific meetings, such as the International Neurotrauma Meeting, various Gordon Conferences, etc., are requested in the amount of $2,000 per year per investigator. A total yearly request is made for Drs. Dore-Duffy and Kreipke in the amount of $4,000.

Other Expenses

Animal costs (purchasing and housing) – Our studies require long-term monitoring of animals in the hypoxic chamber and animal housing. Animals will be housed to acclimate them for 48 hours before inclusion in the studies. In some experiments, mice will be injured using the weight drop model described in the protocol and then housed for varying periods of time up to three weeks before onset of therapeutic intervention. During periods in which animals are experiencing normobaric hypoxia or normoxia, they will be housed in the laboratory and then returned to Wayne State University's D.L.A.R. animal care facilities, in some cases. A request has been made to take in consideration of nonbillable periods. Only 20 of an estimated 25 experiments have been calculated in the request for funds. The number of animals equals 20 experiments **X** 9 time points **X** 4 animals per time point determined by power analysis to yield differences significant at 95%. This equals a minimum of 720 animals and a minimum of 300 control animals per year at a cost of $22/animal (for 2009). The charges for housing for 21 days/cage (small cages-$1/day, large cages-$2/day) equal $800/month. This amount for housing includes charges for the time that the injured animals are separated into individual cages for initial recovery after induction of TBI. A request is made for $35,000, which includes the experimental and control animals for each year and price increases from the animal vendors (e.g., Harlan and Jackson Laboratories) and Wayne State University's Animal Care facilities.

Animal costs (Transgenic) – For our transgenic mouse colonies, we will require 6 large breeding cages and 6 small cages. We require large cages for breeding and small cages to house weanlings, mice for timecourse studies, and to temporarily maintain retired breeders. The cost of animal husbandry in the D.L.A.R. facility at Wayne State University in 2009 will be

$2/day (large cages) and $1/day (small cages). These costs increase by 4% in January of each year; injections of 2 transgene constructs per year into male pronuclei of fertilized embryos. Husbandry costs will be reduced progressively to offset increases in salary.

Equipment Repair/Service contracts – We contribute for service and repair contracts on major laboratory equipment in the Neurology Department, since it is considered "community" equipment. This includes centrifuges, autoclaves, water purifiers, fluorescent microscopes, etc. Each laboratory contributes approximately $1,500 per year. The service contract for the BioSpherix equipment is $3,000 for the hypoxic chambers.

Publication costs – We request funds to help defray the cost of publications. The amount of $2,500 per year will help offset the increasing cost of journal submissions, color images, and reprints.

MR Imaging costs – We will image 50 animals each year by Wayne State University's MR Imaging Facilities. The reduced hourly rate of $150 applies to Federal Grants and Contracts; therefore, for 50 animals (each animal takes approximately one (1) hour to scan), will cost $7,500.00 for the first year.

Shipping expenses – A request is made for $500.00 yearly for the cost of shipping various harvested samples to Cleveland, Ohio to our collaborator, Dr Joseph LaManna.

Software costs – For our image analyses and preparations, we maintain upgrades and site licensing on Adobe Creative Suite (including PhotoShop and Professional); for our statistical analysis, we maintain upgrades and site licensing on SyStat. These software packages are critical for our project and will cost $2,000 per year. Also, the long-term storage, preservation, and management costs are included for maintaining data that we obtain from this proposal and its experiments.

RESEARCH & RELATED BUDGET - Cumulative Budget

		Totals ($)
Section A, Senior/Key Person		490,281.00
Section B, Other Personnel		519,669.00
Total Number Other Personnel	15	
Total Salary, Wages and Fringe Benefits (A+B)		1,009,950.00
Section C, Equipment		62,150.00
Section D, Travel		21,237.00
1. Domestic	21,237.00	
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		615,858.00
1. Materials and Supplies	522,951.00	
2. Publication Costs	13,273.00	
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	39,817.00	
9. Other 2	23,891.00	
10. Other 3	15,926.00	
Section G, Direct Costs (A thru F)		1,709,195.00
Section H, Indirect Costs		856,463.00
Section I, Total Direct and Indirect Costs (G + H)		2,565,658.00
Section J, Fee		

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

1. Project Director / Principal Investigator (PD/PI)

Prefix: Dr.
* First Name: Paula
Middle Name:
* Last Name: Dore-Duffy
Suffix: Ph.D.

* New Investigator? ☒ No ☐ Yes

Degrees:

2. Human Subjects

Clinical Trial? ☒ No ☐ Yes

* Agency-Defined Phase III Clinical Trial? ☐ No ☐ Yes

3. Applicant Organization Contact

Person to be contacted on matters involving this application

Prefix: Mrs.
* First Name: Gail
Middle Name:
* Last Name: Ryan
Suffix:

* Phone Number: 313-577-1445
Fax Number: 313-577-1348
Email: orspsmail@wayne.edu

* Title: Director

* Street1: 5057 Woodward Avenue
Street2: Suite 6402
* City: Detroit
County: Wayne
* State: MI: Michigan
Province:
* Country: USA: UNITED STATES
* Zip / Postal Code: 48202

Tracking Number:GRANT10340434 Funding Opportunity Number:PA-08-015 Received Date:2009-06-05T10:42:03-04:00

PHS 398 Cover Page Supplement

4. Human Embryonic Stem Cells

* Does the proposed project involve human embryonic stem cells? ☒ No ☐ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://stemcells.nih.gov/registry/index.asp. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s): ☐ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Close Form | Print Page | About

OMB Number: 0925-0001

PHS 398 Research Plan

1. Application Type:

From SF 424 (R&R) Cover Page and PHS398 Checklist. The responses provided on these pages, regarding the type of application being submitted, are repeated for your reference, as you attach the appropriate sections of the research plan.

*Type of Application:

☒ New ☐ Resubmission ☐ Renewal ☐ Continuation ☐ Revision

2. Research Plan Attachments:

Please attach applicable sections of the research plan, below.

1. Introduction to Application		Add Attachment	Delete Attachment	View Attachment
(for RESUBMISSION or REVISION only)				
2. Specific Aims	1244-2 specific aims.pdf	Add Attachment	Delete Attachment	View Attachment
3. Background and Significance	1245-3 bg and signf.pdf	Add Attachment	Delete Attachment	View Attachment
4. Preliminary Studies / Progress Report	1246-4 prelim data.pdf	Add Attachment	Delete Attachment	View Attachment
5. Research Design and Methods	1247-5 strategies.pdf	Add Attachment	Delete Attachment	View Attachment
6. Inclusion Enrollment Report		Add Attachment	Delete Attachment	View Attachment
7. Progress Report Publication List		Add Attachment	Delete Attachment	View Attachment

Human Subjects Sections

Attachments 8-11 apply only when you have answered "yes" to the question "are human subjects involved" on the R&R Other Project Information Form. In this case, attachments 8-11 may be required, and you are encouraged to consult the Application guide instructions and/or the specific Funding Opportunity Announcement to determine which sections must be submitted with this application.

8. Protection of Human Subjects		Add Attachment	Delete Attachment	View Attachment
9. Inclusion of Women and Minorities		Add Attachment	Delete Attachment	View Attachment
10. Targeted/Planned Enrollment		Add Attachment	Delete Attachment	View Attachment
11. Inclusion of Children		Add Attachment	Delete Attachment	View Attachment

Other Research Plan Sections

12. Vertebrate Animals	1248-vertebrate animals o6o	Add Attachment	Delete Attachment	View Attachment
13. Select Agent Research		Add Attachment	Delete Attachment	View Attachment
14. Multiple PI Leadership Plan		Add Attachment	Delete Attachment	View Attachment
15. Consortium/Contractual Arrangements		Add Attachment	Delete Attachment	View Attachment
16. Letters of Support	1249-LaManna collab ltr May	Add Attachment	Delete Attachment	View Attachment
17. Resource Sharing Plan(s)		Add Attachment	Delete Attachment	View Attachment

18. Appendix Add Attachments | Remove Attachments | View Attachments

List of Research Plan Attachments

SPECIFIC AIMS

Significance: An alarming number of civilians and military returning from Iraq suffer long term consequences from traumatic brain injury (TBI). While improved protective equipment has greatly reduced the number of sever open head injuries, there is still a rising source of casualties with mild to moderate TBI particularly in the military population. The Independent Study released in January 2004 by the Department of Veterans Affairs states that TBI may account for up to 20% of surviving casualties (1). The U.S. Department of Health and Human Services reports that nearly two-thirds of injured U.S. soldiers sent to Walter Reed Army Medical Center were diagnosed with TBI. Estimates published by the AEI-Brookings Joint Center for Regulatory Studies place the cost for lifetime care at $600,000 to $5 million per individual (2). Direct and indirect costs, including lost productivity totaled roughly $60 billion annually (3). The high cost of medical support, as well as the devastating effect such injuries produce in American family dynamics, justifies and encourages the development of more efficacious and affordable therapeutic interventions.

Despite considerable knowledge of the primary pathophysiological events associated with TBI (4-8), therapeutic efforts are still disappointing. This may be due to a lack of fundamental knowledge on how to maintain CNS homeostasis or to buffer the brain from secondary injury following TBI. Maintenance of CNS homeostasis is the result of a coordinated effort between the cellular constituents of the neurovascular unit (9). Together these cells make fine tuned regulatory adjustments that promote survival and maintain the balance between oxygen and glucose availability and tissue metabolic demand. This involves structural changes that increase capillary density and decrease capillary distance. Such changes underlie physiological or adaptive angiogenesis (10,11). Increased capillary density is seen following exposure to chronic mild hypoxia (10), sensory or motor training (12,13-16) and exercise (14). **Physiological/adaptive angiogenesis has also been referred to as therapeutic angiogenesis and may have potential in clinical management of TBI.**

Together our research team has observed and characterized the angiogenic response following TBI (17-19) adding to a growing body of literature published by other investigators (21-24). We have reported that adaptive angiogenesis may be dysregulated following TBI (18,23,25). Results suggest that although aspects of angiogenesis are induced following TBI, the HIF-1α response is suboptimal, vascular remodeling is ineffective and the balance between metabolic need and tissue oxygen and glucose availability is disrupted (26-28). **We hypothesize that chronic mild normobaric hypoxia-induced physiological/adaptive angiogenesis will stabilize the HIF-1 response, restore metabolic homeostasis, augment neuroprotection and CNS repair, mitigating the extent of secondary injury and sparing cognitive deficit following TBI. To test this hypothesis we address the following specific aims:**

Specific Aim 1. Characterize angiodynamics, neuronal injury and cognitive deficits following TBI. Aim #1 is designed to characterize baseline angiodynamics **(according to the criteria outlined in Table 1)** and correlate these changes with neuronal injury and cognitive outcome following TBI. Analyses will be conducted temporally and serially, focusing on primary cognitive centers of the brain, sensorimotor cortex (smCx) and dorsal hippocampus (hipp), as well as corpus callosum (CC). This aim will provide a sound basis for the experiments outlined in the subsequent specific aims.

Specific Aim 2. Determine the effect of chronic mild normobaric hypoxia on angiodynamics and neuropathologic/cognitive outcome following TBI. We will determine whether therapeutic angiogenesis induced by exposure to chronic mild normobaric hypoxia will lead to improved angiodynamics, neuropathologic and cognitive outcomes following TBI. Exposure to hypoxia will be initiated at varying times following impact. Treatment paradigms will be sustained for periods of up to 4 weeks.

Specific Aim 3. To determine the mechanistic underpinnings of neuroprotection and stabilization of angiodynamics produced by normobaric hypoxia. Aim #3 will identify the mechanisms by which induction of therapeutic angiogenesis is neuroprotective. We will assess the effect of pharmacologically induced angiogenesis or the effect of inhibitors/antagonists targeted to HIF-1α gene expression and stabilization in wild type and transgenic mice. We generated transgenic mice that harbor a tamoxifen-responsive Cre-recombinase ($CreER^{T2}$) under regulatory control of a hypoxia sensitive promoter/enhancer [*EMH-$CreER^{T2}$:ROSA26* mice]. These mice will be used to Fate Map HIF-1 responding cells.

INTRODUCTION/SIGNIFICANCE: An alarming consequence of TBI is long-term neurological and cognitive impairment. A conservative estimate places casualties at 5.3 million Americans (Centers for Disease Control) and accounts for up to 37,000 hospitalizations and 435,000 emergency room visits annually. The high cost of medical support for those afflicted with permanent and secondary distress from TBI, as well as the devastating affect such injuries produce in the American family points to the need to develop more efficacious and affordable therapeutic interventions. In this application we propose to examine the effect of **therapeutic angiogenesis induced by exposure to chronic low levels of hypoxia (HYP).** The proposed treatment paradigm is inexpensive and noninvasive; it can easily be incorporated into treatment strategies in TBI.

Angiodynamics and bioenergetic homeostasis: The maintenance of brain homeostasis and vascular hemostasis is the result of a coordinated effort by the cellular constituents of the neurovascular unit (7). Together the cells make fine-tuned regulatory adjustments that result in adaptation to local and even systemic stress conditions. These adaptations are responsible for maintaining the balance between oxygen and glucose availability and metabolic demand. In the brain there is a direct relationship between capillary density, cerebral blood flow and neuronal metabolic demand. In the adult animal these mechanisms reflect a continuous matching of tissue oxygen with capillary density. As such, structural adaptations that result in increased vascular density have been shown to underlie adaptive (physiological) angiogenesis (10,11). **Maintenance of capillary density in the adult is ongoing and tightly regulated. Dysregulation resulting in the loss of bioenergetic homeostasis may lead to loss of oxygen and glucose delivery and development of pathological conditions associated with neuronal loss and/or axonal injury following TBI.**

Following diffuse brain injury there is a rapid and pronounced vascular response that results in a state of vasoconstriction and diminished perfusion to the brain parenchyma (15-17). This decrease in cerebral blood flow is followed by additional changes associated with vascular adaptation suggesting that the brain has initiated aspects of physiological AG in response to hypoxic stress. Our laboratory has observed and characterized elements of the angiogenic response following TBI (18) adding to a growing body of literature published by other investigators (20-24). We have reported that vascular function (23,25) and adaptive AG may be dysregulated in TBI (18). Dysregulation is particularly evident in CNS pericytes (PC). Even following mild TBI, PC function is altered (18,23). One of the earliest responses to TBI is the migration of PCs from their vascular location (23). PCs that migrated were protected from injury while those non-migrating PCs within the same capillary or nearby microvessels became apoptotic (23). Abnormal PC physiology in TBI is supported by our observation that the VEGF/ HIF-1α response is abnormal in cortical capillaries (18). A depressed HIF-1 response in TBI has been confirmed by others (29-31). As PCs are intimately involved in the regulation of vascular function and AG (32-34), results suggest that adaptation to injury at the vascular level may be abnormal. **Taken together studies indicate that while the angiogenic response is induced following TBI, it is suboptimal and vascular remodeling is ineffective and not capable of maintaining metabolic homeostasis. Dysfunctional angiodynamics is deleterious to both acute and long term clinical outcome as the adaptive response is insufficient to protect the brain from neuronal death and cognitive damage post TBI. We hypothesize that restoration of metabolic homeostasis through induction of therapeutic AG will mitigate the extent of secondary injury sparing cognitive decline following TBI. In the present application we propose to test the effect of therapeutic and behavioral intervention that promotes physiological adaptive AG and restoration of the HIF-1 response on clinical outcome and long term recovery following TBI.**

Adaptive angiogenesis (AG) and hypoxia (HYP): In the adult, adaptive angiogenic responses have been observed in areas of the brain associated with motoric and behavioral functions (35-36). AG is induced following learning (36,37), exercise (12-14) and following chronic exposure to mild HYP (11,38,39). We have further shown that exposure to normobaric HYP is neuroprotective in an animal model of chronic CNS inflammation (40). Exposure to hypobaric HYP (FiO_2=0.10, 10%) (38) or to normobaric HYP (6-15% O_2) results in a doubling of capillary density by two to three weeks. Increased vascular density following exposure to chronic moderately low oxygen is also seen naturally in animals or humans exposed to high altitude (38,39). Adaptive AG is beneficial and promotes tissue survival. **The beneficial response to low oxygen that is physiological is in sharp contrast to the deleterious effects of HYP observed following exposure to acute anoxic levels of HYP, superimposed HYP/ischemia or to the effects of low oxygen during the acute phase of brain injury.**

The apparent paradox: How can one reconcile the seeming paradox between the adverse effects verses beneficial effects of HYP? Multicellular organisms have developed highly complex mechanisms by which they maintain optimal tissue oxygen levels. Under conditions of stress or injury such as TBI there is a coordinated series of systemic adaptations, acute adaptations and more profound structural adaptations that promote survival (11,38,39). PC function is thought to be essential to these adaptations (11). If vascular

adaptive responses are inadequate to restore bioenergetic homeostasis there is disequilibrium between metabolic need oxygen tension and glucose utilization and ultimately neuronal injury/loss. Loss of bioenergetic homeostasis is a "harbinger" of poor outcome after TBI (41-45), giving credence to the supposition that tissue oxygen should be monitored in patients following severe TBI (44-51). That tissue oxygen is compromised following TBI is an accepted concept and cerebral oxygenation is routine standard care (46,50). Superimposing severe HYP or HYP/ischemia during the initial traumatic insult has been shown to exacerbate the injury process in both humans and animals (47,51,52). In cases of hemorrhagic hypotension there is sustained reduction in oxygen delivery in the brain despite normal systemic oxygen delivery (53). Similar results have been reported in animals (54-58). It is important to consider the **magnitude** of HYP, the **rapidity of exposure** (rapid exposure to 10% O_2 is less tolerated), the **duration of exposure**, the **age of the patient**, and most importantly the **coexisting clinical factors** in each patient. Anoxia verses conditions of HYP without ischemia is more detrimental (59). Recent studies have shown that anoxia induces a substantially different genetic response than HYP (60,61). Differential responses to anoxia verses graded HYP can be cell specific (59). Following periods of anoxia, adult neurons lose a large amount of their intracellular K+ resulting in an 8-fold increase in extracellular K+ (62). This is due to activation of K+ channels. This was not seen under conditions of varying oxygenation. Similar dose related effects have been observed in muscles (63), liver (64), and mesangial cells of the kidney (65). **Thus, severity or degree of HYP is important and directly affects the final outcome.**

In addition to severity, **speed or rapidity** of onset also has a profound effect on clinical outcome. In the case of altitude sickness, where HYP develops gradually, symptoms include headaches, fatigue, shortness of breath, a feeling of euphoria and perhaps nausea. These symptoms are generally transient. In severe HYP, or HYP of very rapid onset, changes in levels of consciousness, seizures, coma and death may occur. Rapid exposure to low oxygen (FiO2 $\leq$ 0.1) for varying periods of time may lead to transient changes or permanent changes, depending on the severity of hypoxic insult, in a number of performance parameters (66). This has lead scientists and physicians to recommend **graded, gradual exposure to mild HYP in a slow stepwise manner (67). This makes the hypoxic stimulus more easily tolerated due to induction of adaptive mechanisms (10). In this application we will use a graded exposure to mild HYP.**

The **duration of the hypoxic stimulus** may also induce fundamentally different reactions. For example, rapid intermittent exposure to HYP can induce hypoxic preconditioning (68-70). Short term intermittent exposures are well tolerated (60) but long term intermittent exposure can lead to complications (71,72). **Chronic exposure to low levels of oxygen is well tolerated and leads to adaptive AG (10,38).** However, adaptation to even mild HYP may affect the young differently than the aged. It has been well accepted that young animals are more resistant to hypoxic damage than aged animals. In response to chronic mild HYP aged animals do not mount an adaptive response (38; Dore-Duffy and LaManna unpublished observations). Similarly aged patients have poor outcome from TBI (73). This may be due to a diminished HIF-1 response to HYP. Stabilization of the HIF-1α response underlies neuroprotection (74).

Confounding alterations in angiodynamics may exacerbate the effects of even mild HYP. Effects of systemic blood pressure, $PaCO_2$, PaO_2, and cerebral edema on cerebral blood flow and oxygenation are relatively well known (11,75-78). Moppett and Hardman (42) proposed a computational model to quantify effects or interactions between these factors. Significant cerebral ischemia was unlikely to occur with isolated physiological changes according to their model. However, the combination of two or more factors such as hypotension, edema and/or HYP made ischemia much more likely in this model. Even mild HYP superimposed on a clinical background associated with factors that compromise oxygen delivery is likely to have a deleterious effect and must be considered in the interpretation of data.

In conclusion the role of HYP in the angiodynamics following TBI is complicated. Our studies indicate that the angiogenic response is induced following TBI and that vascular remodeling involving PCs may be ineffective and not capable of maintaining metabolic homeostasis. The early HIF-1 response in PCs within hrs of TBI may be associated with the induction of apoptosis in these cells. HIF-1 induced by reactive oxygen species may be deleterious to survival. Dysfunctional angiodynamics will have a profound deleterious effect on both acute and long term clinical outcome as the adaptation is insufficient to protect the brain from neuronal death and cognitive damage post TBI. We hypothesize that restoration of metabolic homeostasis and angiodynamics will mitigate the extent of secondary injury sparing cognitive decline following TBI. In the present application we propose to test the effect of therapeutic and behavioral intervention that promotes physiological adaptive AG and stabilization of the HIF-1α response on clinical outcome and long term recovery following TBI. Our proposal will extend work by our laboratories characterizing physiological adaptive AG in a rodent

acceleration impact model (modified Marmarou model). We will determine the extent of neuronal damage and cognitive and biobehavioral outcome following injury with and without therapeutic HYP. In addition we will test possible mechanisms underlying neuroprotection.

PRELIMINARY DATA

RESULTS FOR SPECIFIC AIM#1: Characterize angiodynamics, neuronal injury and cognitive deficits following TBI. There are many validated models for inducing TBI that reproduce injuries that include open-head trauma with significant brain parenchymal bleeding, immune sequelae as well as nerve cell death. Fluid percussion (FP) and cortical impact (CI) models, for example, involve exposing the cortex to direct focal contusion. While FP and CI have been used extensively to study the effects of TBI, the severity and nature of damage do not directly relate to the type of head injury incurred by the majority of civilians and military personnel. An acceleration impact model developed by Marmarou (78) produces diffuse axonal injury (DAI), impaired vasoreactivity, perivascular edema and increased intracranial pressure with little or no parenchymal bleeding or its post-inflammatory sequelae. While the initial model had certain shortcomings, the **Modified Marmarou model** that incorporated a steel helmet for force displacement, better alignment of the impactor to the helmet to reduce the risk of bilateral asymmetric results and holes drilled along the tube to ensure that wind velocity does not cause resistance or subtle movements in the rat's head, has proved highly beneficial. Significant behavioral deficits have been reported using this model (13,14). We have established the modified Marmarou model in our laboratory for both rats and mice and have published using this models (79,80).

Neuronal injury following TBI: We have examined pathological changes observed at 1 hour, 4 hours, 12 hours and 2 days following TBI. Results are shown in **Figure 1**. As early as one hour post-injury, we observed the presence of large retraction bulbs in relation to corticospinal tract fibers and at the pontomedullary junction. At 4 hours and greater survival times the number of bulbs and axonal fragments increased. Evidence of axonal fragmentation first appeared in hypothalamic nuclei. Evidence of fragmentation appeared in the cortex, corpus callosum and hippocampus at later time periods and may be associated with vascular changes.

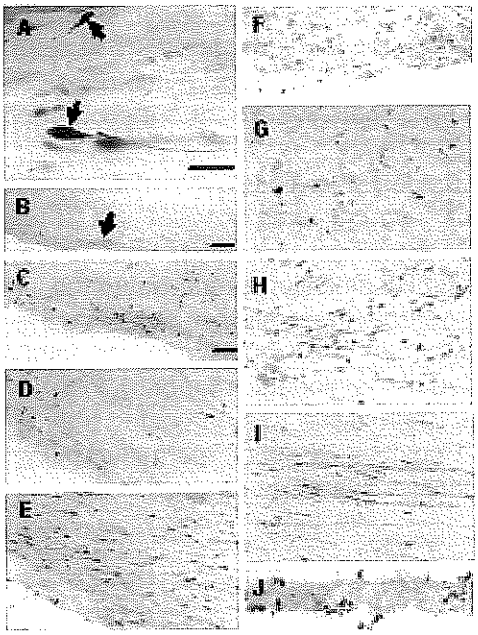


Figure 1. Photomicrographs of amyloid precursor protein (in sagittal (A-E) and cross sections (F-J) from rat brains at 1 h (A, B, F), 4 h (C), 12 h (D) and 48 h (E, G-J) after traumatic brain injury (TBI). Scale in B equals 5.0 µm. Scales in B and C measure 0.5mm and 200µm, respectively. Scale in C is also applicable to D-J. As early as 1 hr post TBI, presence of large (4.0-5.0µm) retraction bulbs can be detected in relation to corticospinal tract fibers (A, arrows) at the pontomedullary junction (B, arrow and BP, brachium pontis). With increasing survival time (4-48 h post TBI), the number of bulbs and axonal fragments are noticeably increased (D-F). Early ontogeny of axonal fragmentation is also detected in hypo-thalamic nuclei (F), while that in cerebral cortex (G), corpus callosum (H), and hippocampal commissure (I) appears to occur at later survival times. Large pial vessel with numerous retraction bulbs and axonal fragments surrounding its wall (J) and not observed in relation to brain parenchymal vessels.

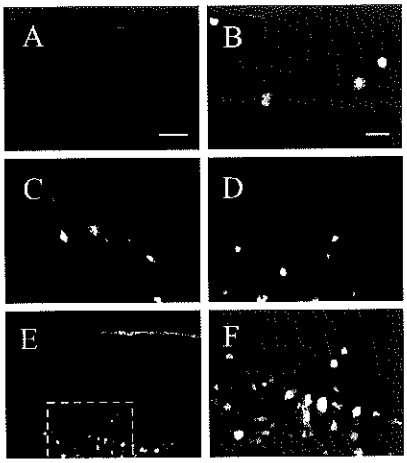


Figure 2. A and B-F depict Fluoro-Jade (FJ) stained cross sections through the upper layers of the sensorimotor cortex below the impact from brains of sham-operated (A), 15 min (B), 4 h (C), 24 h (D) and 48 h (E,F) after TBI. Inset in E is reproduced at a higher magnification in F. Scale in A equals 50µm and applies also to E. Scale in B equals 20µm and is also applicable to C-F. While no FJ labeled neurons are observed in sham- operated brains (A), increasing numbers of FJ labeled neurons are evident in layers II-III of the cortex at 4 h, 24 h and 48 h post TBI (C, D, E, respectively). The size and overall morphology of the labeled neuronal cell bodies are consistent with those small pyramidal neurons in layers II

We have assessed the extent of neuronal damage following TBI using FluoroJade techniques as described in general methods. **Figure 2** shows results of a sham operated (control) animal versus animals analyzed at varying times post injury. Results show that evidence of neuronal injury augments with time.

Cognitive Deficits Following TBI: Results shown in **Figure 3** using an eight-arm radial maze test establish that the latency in completing the task is significantly increased following trauma. Experimental animals consistently performed at or around the maximal allotted time, 10 min for up to 14 days. Performance gradually improved over time only approaching control levels after 25 days post impact.

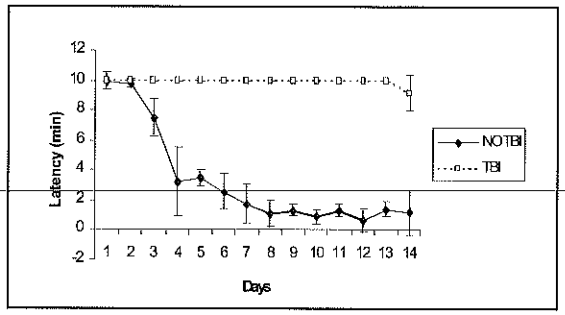


Figure 3. TBI causes cognitive deficits. Naïve control (No TBI) and experimental (TBI) animals were introduced to the eight-arm radial arm maze. Control animals achieved baseline performance (defined as completing the test in 3 min or less) in approximately 6-7 days. Experimental animals consistently performed at or around the maximal allotted time, 10 min. Statistical comparison revealed that experimental animals performed significantly longer at days 3-14.

Angiodynamics following TBI

Angiogenesis is the process by which new vessels form from an existing vascular bed and in the adult is thought to be an endogenous adaptive mechanism to increase oxygen and glucose delivery to the tissues. Induction of angiogenesis is protective and ameliorates the deleterious effects of hypoxia on neurons in stroke models (81). In a transient middle cerebral artery occlusion (MCAO) model of stroke, VEGF administration was shown to decrease infarct size and improve neurologic outcome (82). In the same model, there is sparing of neuronal apoptosis (81). In a focal thromboembolic model of stroke, interaction of two VEGF isoforms (VEGF A and C) was shown to promote angiogenesis and suggested to participate in subsequent spontaneous reperfusion (83).

In TBI angiogenic factors have been shown to be upregulated in models of brain trauma. A study using a cortical impact model of TBI provided evidence of angiogenesis, including upregulation of VEGF, VEGFR1 and VEGFR2 in and around the lesion site (84,85). This same study reported an increase in vascularization around the site of injury (85). Importantly, the same group found that VEGFR2 inhibition exacerbated the extent of cell injury following cortical impact. Collectively, the data reported from these studies suggests that elements of the angiogenic response are induced following TBI and may be part of an adaptive mechanism to promote tissue repair and survival. In our model of TBI we have evaluated the angiogenic response following TBI. Results indicate that vascular density is increased following impact. Brain tissue was harvested after 21 days following injury. In **Figure 4** (left) we show that the density of glut-1 positive vessels [determined using stereological techniques] is increased following TBI. Increased vessel density was also observed in the hippocampus (hipp) and smCX (right graph).

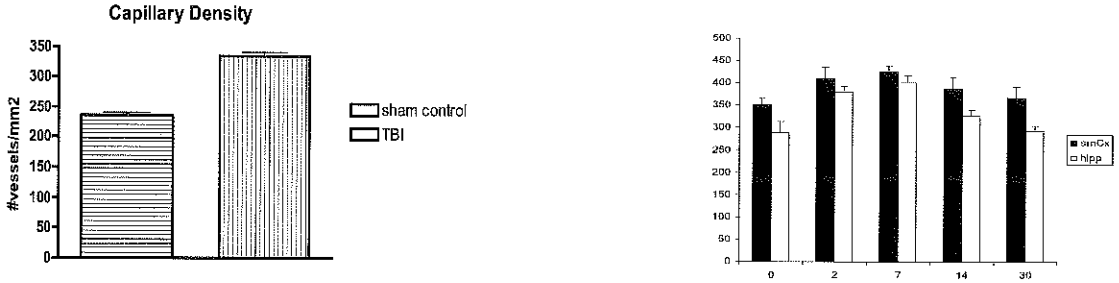


Figure 4. Capillary density following TBI. In two separate studies, glut-1 positive vessels were calculated by stereology after 21 days and vessel density graphed. In the second set of experiments, vessel density was determined in the smCx and hipp (right). Results indicate that angiogenesis was only moderately increased following TBI.

In a second series of experiments capillaries were isolated from impacted brain tissue at varying times following injury immediately following sacrifice. Capillaries were stained for immunologically reactive proteins involved in regulation of physiological angiogenesis. The percentage of vessels expressing greater than

background fluorescence was determined by image analysis. Nonspecific isotype control antibody and secondary antibody alone was used to determine background. Results are graphed in **Figure 5** and compared to that observed following hypobaric hypoxia induced induction of physiological angiogenesis. Results indicate that the angiogenic response to TBI is suboptimal. Additional comparative data is discussed below in results for Specific Aim #2.

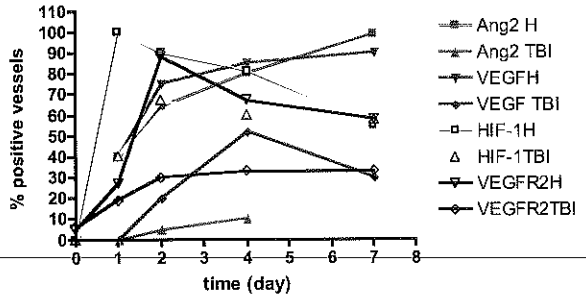


Figure 5. Capillaries were isolated from cortical tissue at various times following impact or exposure to hypoxia. Vessels were stained for expression of the indicated antigen. The percentage of vessels expressing relative fluorescence intensity of above background fluorescence obtain using nonspecific antibody and appropriate second antibody controls quantified using image J software. Results indicate that the angiogenic response following TBI is substantially less than seen following exposure to chronic mild hypoxia.

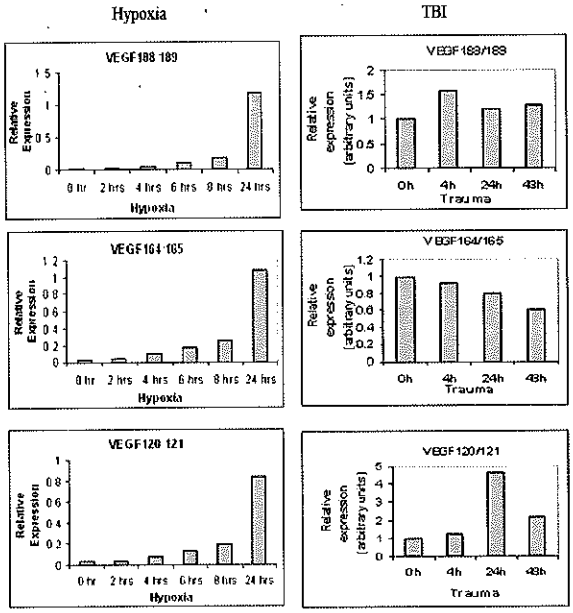


Figure 6. Capillary VEGF isoforms specific transcripts. Animals were exposed to hypoxia or to TBI and animals were sacrificed at varying times. Cortical capillaries were isolated immediately and isoforms specific mRNA quantified by real time PCR. Results indicate that the major isoforms produced following TBI is VEGF 120/121 (representative experiment).

We also examined capillary expression of vascular endothelial growth factor (VEGF). Freshly isolated capillaries were processed for total messenger RNA (mRNA) at various times following injury. VEGF isoform specific transcripts were quantified by real time polymerase chain reaction (RT-PCR). Similar analysis was performed on freshly isolated capillaries following exposure to hypoxia. Results are graphed in **Figure 6**. Data indicated that following exposure to chronic mild hypoxia capillaries are induced to express VEGF specific transcripts for the classic isoforms VEGF 120/121, VEGF 1664/165, and VEGF 188/189 whereas following TBI, only the VEGF 120/121 isoform specific transcript was induced ($P \leq 0.01$). Further, the capillary HIF-α response to TBI is significantly less than that observed following exposure of animals to low oxygen (**Figure 7**). These results suggest that there may be deregulation of the angiogenic response following TBI. While the smaller VEGF isoforms has been shown to induce new vessel formation it is thought that VEGF120 has a primary role in cell differentiation. As the capillary PC has been shown to be the earliest cell to express VEGF following hypoxic stress (11) or following TBI these results suggest that compromised pericyte function may contribute to loss of metabolic homeostasis following TBI.

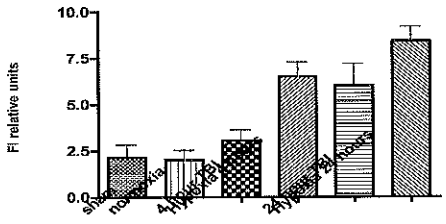


Figure 7. HIF-1α protein expression following TBI. Capillaries were isolated from brain tissue immediately following sacrifice at the indicated times following TBI or hypoxia. HIF-1α was determined by immunocytochemistry and quantified by image analysis using Image J software. Relative units = mean +/- SD. FI= fluorescence intensity.

RESULTS FOR SPECIFIC AIM#2: Therapeutic angiogenesis ameliorates injury following TBI.

2.1 Angiodynamics following Exercise Conditioning.

Physiological/adaptive angiogenesis in the brain can be induced in a number of ways: exposure to chronic mild hypoxia, exercise conditioning, and learning. Our laboratories have extensive experience with 1) exercise conditioning and 2) chronic mild hypobaric and normobaric hypoxia. We have shown that both exercise conditioning and exposure to chronic mild hypoxia induces angiogenesis and promotes tissue survival. Physiological/adaptive angiogenesis maximizes the brain's natural buffering capacity against disrupted blood flow and injury and can be used as an approach to therapy. We have begun to look at therapeutic angiogenesis in TBI. **Figure 8** shows that exercise conditioning increases VEGFR2 expression following TBI. Results in **Figure 9** show that exercise conditioning also enhanced vascular density suggesting the induction of angiogenesis.

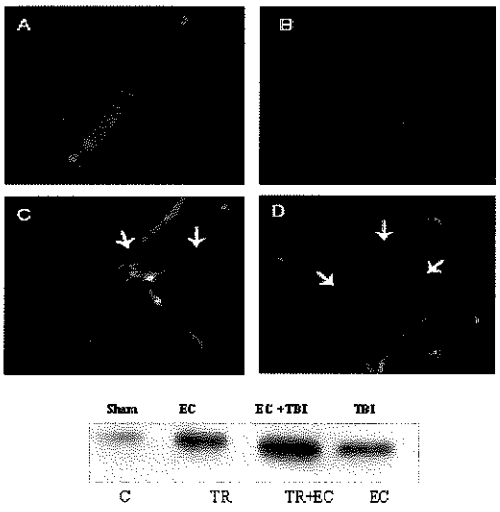


Figure 8. Effects of exercise and trauma on VEGFR2 protein expression and immunofluorescence within endothelium (EN). A) X40 view of EN (FITC, green channel) within smCx showing little to no VEGFR2 immunofluorescence (Texas red, red channel) within control animals of three different areas of the smCx. B) X40 view of microvessels within smCx of conditioned animals showing VEGFR2 expression (arrows) along EN. Note that VEGFR2 immunoexpression appears to be most prominent in discreet compartments adjacent to areas of vessel branching. C) Photomicrograph of VEGFR2 expression (arrows, red channel) within EN 48hr post TBI. As in conditioned animals, VEGFR2 is most prominent in discreet compartments along the EN. D). Microvessels of animals receiving conditioning and trauma showing very intense VEGFR2 immunoreactivity. E) Western analysis of VEGFR2 protein. Note that both exercise preconditioning (EC) and trauma (TR) increase VEGFR2, EC VEGFR2 is higher in smCx. EC+TR increased these effects most prominently (N = 3 per group).

Figure 9. Evidence of angiogenesis following exercise conditioning (EC) + trauma (TR). A-B) Capillary density in smCx of sham operated animals with no exercise conditioning (A) and 48 hrs post TBI (TR) animals in animals that underwent exercise conditioning (EC) (B). Note the increase in vessel branches (arrows) in the EC+TR relative to control. C) Quantitative analysis of capillary density within both smCx and hipp revealed a significant increase in relative to controls. Measurement bar=50 μm.

2.2. Angiodynamics following exposure to normobaric hypoxia: The in vivo model of adaptive angiogenesis in response to chronic mild hypoxia. We have extensive experience with models of physiological angiogenesis and have published using this model. We have an on going collaboration with Dr. Joseph LaManna at Case Western Reserve investigating hypobaric hypoxia. We have also set up the normobaric hypoxia system (Dore-Duffy laboratory). The results of our collaborations have indicated that our in vivo normobaric hypoxia model mirrors Dr. LaManna's in vivo hypobaric hypoxia model. Exposure to mild hypoxia in both models induces a hypoxia driven increase in vascular density (**Figure 10, left**). Both models have been adapted to the mouse (**Figure 10 middle and right**). Hypoxia increased vascular density in the mouse. Tissue HIF-1α is induced in cortical tissue in both mice and rats (**Figure 10**). Mice were exposed to hypoxia for varying periods of time.

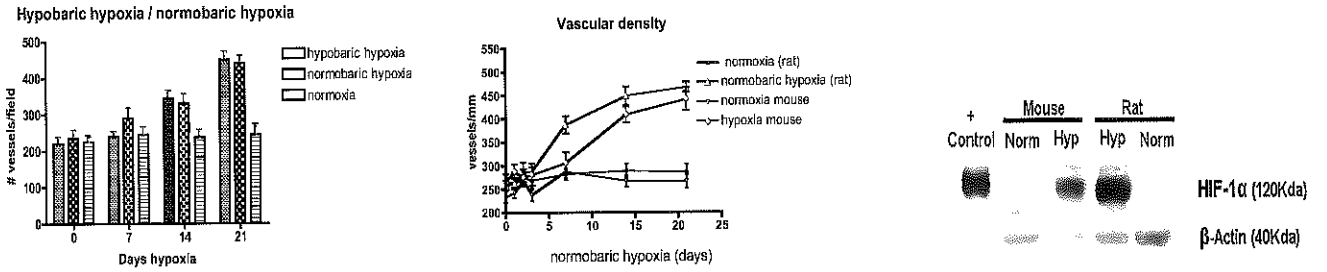


Figure 10. Comparison between the effect of hypobaric hypoxia and normobaric hypoxia. Comparison between rats and mice. Animals were exposed to hypoxia (10% O2) for varying periods of time. Animals were sacrificed and thin sections randomly chosen over the cortex. At least 4 sections per area were counted for the number of glut-1+ vessels (top left and right). Both hypobaric and normobaric hypoxia produce comparable increases in vascular density (middle). The response of mice and rats were comparable. The HIF-1α response was comparable at 24 hours between mice and rats shown in a western blot (right).

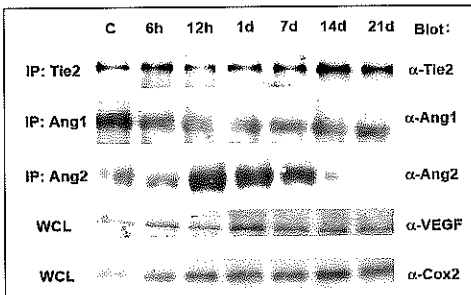


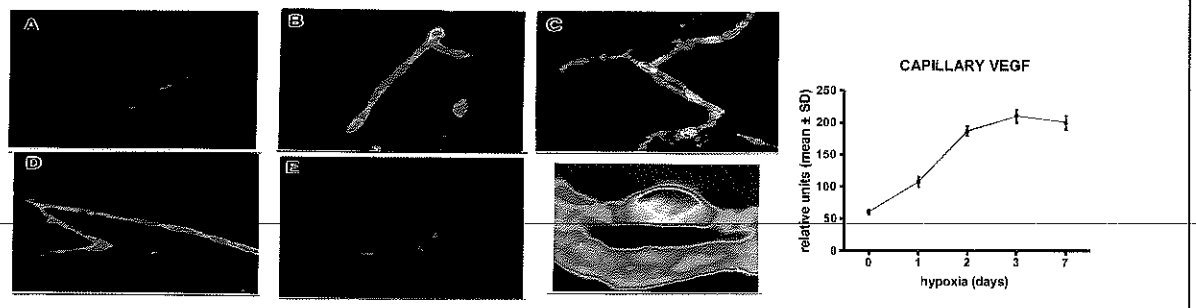
Figure 11. Ang2, VEGF, Tie2 and Cox2 protein is increased in C57BL/6 mouse isolated microvessels exposed to hypoxia. Whole cell lysates (WCL) from mouse cortex were immunoprecipitated (IP) or run as WCL and then blotted as indicated against Tie2, Ang1, Ang2, VEGF, or Cox2. Tie2 and Ang1 levels did not change from control normoxic cortex, following 6 hours (h), 12 hours, 1, 7, 14, or 21 days. Ang2, VEGF and Cox2 all increased as early as 6 hours following exposure.

Ang-2, VEGF, Tie2 and COX-2 protein were analyzed from immunoprecipitates by western analysis (**Figure 11**). Ang-1 expression in capillaries marginally decreased over the study period. Tie2 protein expression did not significantly change until 14 days when we observed a moderate increase. Ang-2 and VEGF increased as early as 6 hours following exposure to hypoxia. VEGF expression is first seen in the PC and then expression was observed in EC and astrocytes at later time points. By 21 days at maximum capillary density, Ang-2 and VEGF had returned to normoxic levels. COX-2 was increased throughout the study period (**Figure 11**). These results indicate that angiodynamics is altered in response to chronic mild hypoxia.

Capillary VEGF. Capillaries were isolated from animals exposed to hypoxia for varying periods of time and stained for expression of immunologically reactive VEGF165. Results in **Figure 12** indicate that VEGF protein is observed as early as 4 hours following exposure to hypoxia. Early protein appeared to be associated with round nuclei comparable to an association with capillary pericytes (11). At later times immunologically reactive VEGF appeared to be associated with the entire capillary. We feel that this is due to the uptake of VEGF by EC or the redistribution of VEGF throughout pericyte projections. In our hands EC do not express VEGF in response to hypoxia but isolated pericytes do synthesize this protein following hypoxic stress (data not shown).

2.3. The effect of therapeutic angiogenesis on neuropathologic/cognitive outcome following TBI.
We have shown that there is decreased cognition following TBI as assessed on a radial arm maze (discussed above in **Figure 3**). Figure 3 showed the results of a 30-day study in which cognition was measured daily in both sham operated and TBI-induced animals. Results indicate that decreased performance on the radial arm maze is induced in this model of TBI. Exercise conditioning significantly enhanced performance on the radial arm maze (**Figure 13)**. Similar results are seen following exposure to chronic mild hypoxia. Evidence of recovery was seen a full 3 weeks prior to natural recovery observed in untreated animals

Figure 12 .VEGF expression in microvessels from animals exposed to hypoxia is shown below. Microvessels were isolated at 0 (A), 24 hours (B), 48 hours (C), 3 days, 7 days (D) and 21 days (E) when hypoxia induced angiogenesis is complete. Capillaries were stained with antibody directed against VEGFA. The enlarged photograph represents a close up of a pericyte in the vessel. Fluorescence intensity (FI) was quantified by image analysis (right).



In addition, we questioned whether increased angiogenesis was associated with improved blood flow. We first determined the extent of hypoperfusion following TBI using MRI as a non-invasive technique [Dr. Christian Kreipke has been trained in neuroimaging techniques]. We have recently published data showing a nearly 30% reduction in blood flow as assessed using susceptibility weighted imaging (SWI) and anterior spin labeling (ASL) (86). We will employ the same techniques to assess blood flow following therapeutic normobaric hypoxia in TBI.

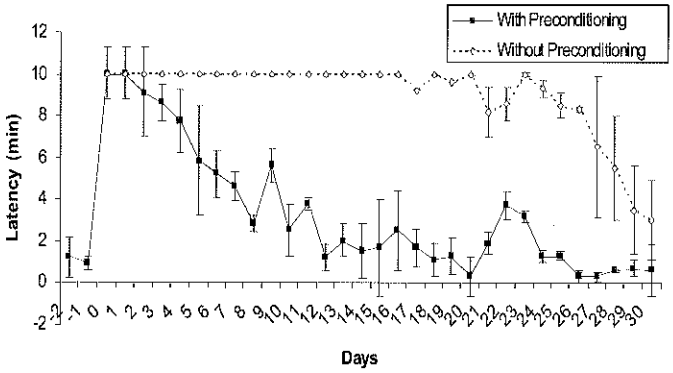


Figure 13. Effect of exercise conditioning on performance on a radial arm maze following TBI. N=6 per group.

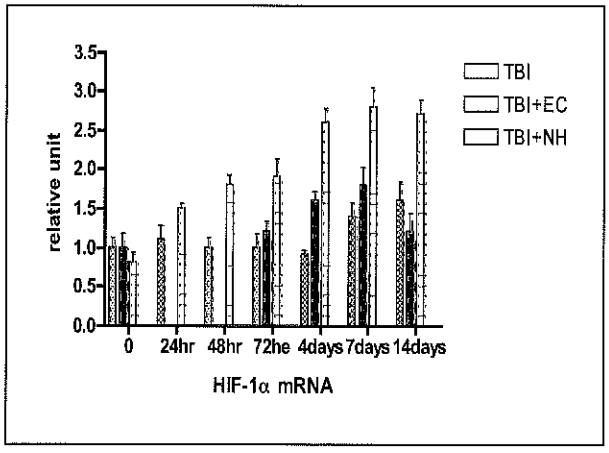


Figure 14. RT-PCR analysis of HIF-1 after TBI (yellow) and after TBI+exercise conditioning (green). Animals were given TBI and then allowed to recover. Post injury animals were either given no further treatment (TBI), exercise conditioning (TBI+EC) or hypoxia for up to 14 days (n=3 per group). At 24, 48 and 72 hours, 4, 7 and 14 days animals were sacrificed, and capillaries isolated. HIF-1α transcripts were quantified by quantitative real time PCR (RT-PCR) analysis. Results show that HIF-1 transcript is increased by 24 hrs hypoxia and 4 days with exercise conditioning as opposed to 7 days with just TBI.

RESULTS FOR SPECIFIC AIM# 3. To confirm the mechanistic underpinnings of neuroprotection and augmentation of angiodynamics produced therapeutic adaptive normobaric hypoxia.

1. Stabilization of the HIF-1 response: One of the mechanisms responsible for therapeutic angiogenesis is thought to involve the stabilization of HIF-1α. Pericytes are the first cell to become HIF-1+ in response to

chronic mild hypoxia (11). We have published previously that pericytes migrate following TBI and that those remaining in their vascular location become apoptotic (23). Loss of pericytes due to migration or apoptotic cell death following TBI would therefore severely compromise the vascular stress response. This may suggest that the HIF-1α response is compromised in TBI. Capillary HIF-1α (**Figure 6,7**) is decreased following traumatic brain injury. It is thought that mitochondria regulate the stability of HIF-1α through the increased production of reactive oxygen species (ROS) (87). Nitric oxide and other inhibitors of mitochondrial respiration prevent the stabilization of HIF-1α during severe hypoxia or anoxia due to an increase in prolyl hydroxylase–dependent degradation of HIF-1α (88). Mitochondrial oxidative damage and dysfunction has been described in focal TBI (89). Results above using two different approaches to the induction of angiogenesis [exercise conditioning and normobaric hypoxia] indicate that induction of angiogenesis increased HIF-1α transcript much earlier than that observed following TBI (**Figure 14**). While this needs to be confirmed by western blots for HIF=1α protein, the results suggest that exposure to chronic mild hypoxia augments and stabilizes the HIF-1α response.

Table 1. Capillary PC:EC ratios following TBI

Time PI (hours)	PC:EC*	% capillary fragments with no pericytes
injured		
0	1:5	4
2	1:5	4
4	1:7	8
24	1:10	22
48	1:12	41
Sham		
0	1:5	3
2	1:5	4
4	1:4	4
24	1:5	5
48	1:5	5

*Number of round nuclei to elongated nuclei microvessel fragment. N=50 fragments per isolation and 2 animals and 2 experiments.

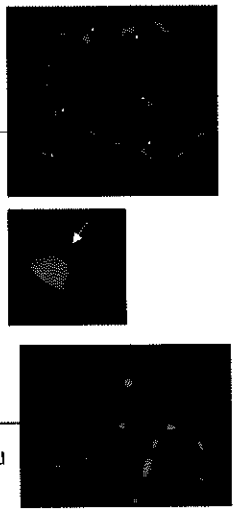
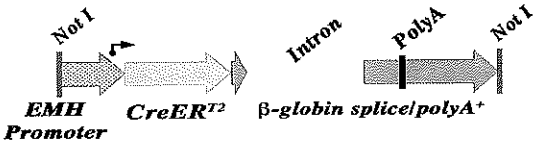
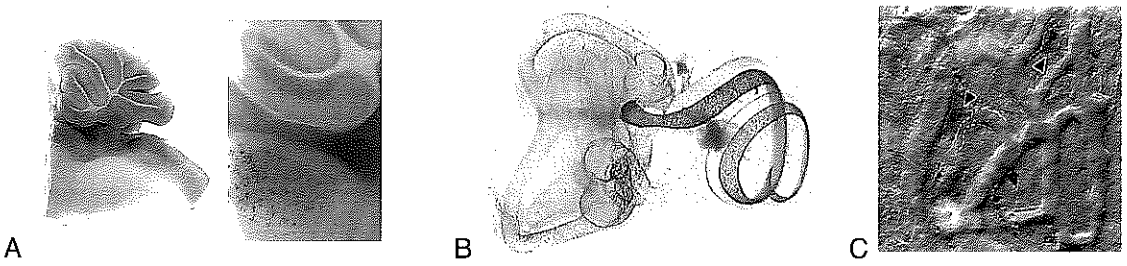


Figure 15. Pericyte (PC) to endothelial cell (EC) ratio following TBI. Capillaries were isolated at various times following TBI. Vessels were stained with DAPI (right). Round nuclei (pericyte) (shown in enlargement) versus elongated cigar shaped nuclei (EC) were counted. The ratio of PC:EC is recorded below. By 24 hours the ratio begins to go down and by 2 days is substantially decreased. Pericytes that do not migrate become TUNEL and by EM are undergoing a degenerative process (lower panel) (23).

2. Pericytes in TBI and EMH-CreER^{T2}:ROSA26 mice: As discussed above pericytes migrate from their capillary location following TBI (23). Non-migrating pericytes undergo apoptosis (23) (**Figure 15**). The pericyte to EC ratio is decreased (**Figure 15**). There is endothelial activation (25) and the pericyte VEGF response is altered (18). As pericytes have an important role in the induction of angiogenesis, compromised pericyte function could blunt the adaptive processes required to maintain bioenergetic homeostasis in the traumatized brain. This may underlie the abnormal HIF-1α response in TBI.

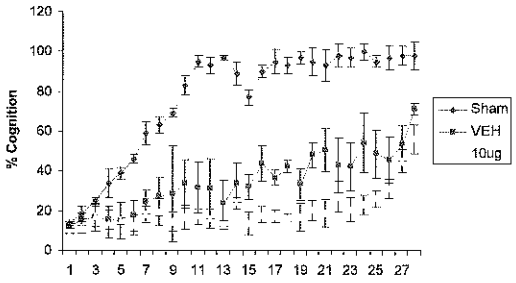


To further investigate this possibility in collaboration with Dr. Alex Gow, we have generated transgenic mice that express Cre recombinase under a strong HIF-1α responsive promoter/enhancer. During hypoxia, *Cre* recombinase is expressed and enters the nucleus if tamoxifen is given. When *Cre* recombinase enters the nucleus it serves as a genetic switch which activates the *ROSA26-lacZ* gene also present in these mice. Thus, cells that respond to hypoxia permanently express beta-galactosidase and can be followed over time in sections and wholemount tissues after histologic and immunofluorescence staining. Pericytes are the first cell to mount a HIF-1 response following exposure to mild hypoxia. Thus the EMH-CreER^{T2}:ROSA26 mice can be used to fate map pericytes. *EMH-CreER^{T2}:ROSA26* mice have been generated on the C57BL/6J background. Preliminary results show reporter gene expression following exposure to as little as 24 hours in 8-10% oxygen. The beta-galactosidase reporter is localized to small perivascular cells in the brain. This confirms results by Dore-Duffy and LaManna that the earliest HIF-1α responding cell is a pericyte.



When the mice are exposed to normobaric hypoxia for one week with daily tamoxifen gavage, beta-galactosidase positive cells are found throughout the brain (shown above **A**). Indeed, we also observe labeled pericytes in most tissues, including the major vascular compartment of the cochlea (**B**) the lateral vascularis (a vascular rich section of the cochlear that contains no astrocytes). Blue cells cupping the microvessels are shown in **C**. These results suggest that pericytes perform similar functions throughout the body. Additional experiments in brain will be needed to confirm pericyte expression of beta-galactosidase.

3. <u>Inhibition of the angiogenic response in TBI</u>. Results discussed above suggest that adaptive angiogenesis may normalize the delivery of oxygen and glucose to injured tissue. These results suggest that therapeutic angiogenesis is protective following TBI. Therefore we reasoned that inhibition of angiogenesis should inhibit the beneficial effects of normobaric hypoxia. To test this hypothesis we conducted experiments using SU5416, a selective VEGFR2 antagonist. Animals received two injections of SU5416 (10μg intracerebro-ventricularly) prior to injury and then were given once daily injections at the same dosage following trauma. Control animals received vehicle (VEH) alone. Treatment was continued for the duration of the behavioral testing. Results in **Figure 16** show that VEGFR2 antagonism inhibited cognitive improvement following injury. This supports a protective effect of angiogenesis following TBI. By inference these results suggest that VEGFRF2 antagonism will also inhibited the improved cognitive outcome following normobaric hypoxia and indirectly supports the supposition that induction of endogenous adaptive angiogenesis will improve clinical outcome following TBI.



<u>Figure 16.</u> The effects of SU5416 on behavioral recovery following TBI. Three groups of animals were used (N=6 per group), Sham operated (blue), TBI + veh (10% DMSO) (pink) and 10μg SU5416, a selective VEGFR2 antagonist. Results indicate that blocking VEGFR2 worsens cognitive recovery following TBI. Data expressed as %cognition, which accounts for latency and number of errors (see General Methods).

RESEARCH STRATEGIES

Rationale: The goal of this proposal is to identify effective, inexpensive, and noninvasive approaches that promote CNS repair following TBI. Our hypothesis is that adaptive repair mechanisms that are geared to maintain bioenergetic homeostasis are compromised following injury and that therapeutic interventions that promote physiological angiogenesis will improve clinical and cognitive outcome. At a basic level the maintenance of brain homeostasis is the result of a coordinated effort by the cells comprising the neurovascular unit (9-11). Together these cells make fine tuned regulatory adjustments that result in adaptation to local and even systemic stress conditions. These adaptive responses are responsible for maintaining the balance between oxygen availability, bioenergetics and tissue metabolic demand. We have evidence that at least two members of the neurovascular unit (pericyte and neuron) are compromised following TBI and that this results in a suboptimal or altered regulation of the HIF-1α response. In the brain there is a direct relationship between local capillary density, cerebral blood flow and neuronal metabolic demand. Mechanisms that increase capillary density (physiological angiogenesis) following injury promote survival. If maintenance of capillary density in the adult is ongoing and tightly regulated then dysregulation resulting in the loss of bioenergetic homeostasis could lead to loss of oxygen and glucose delivery, defective repair and axonal injury following TBI. **We hypothesize that restoration of bioenergetic homeostasis through therapeutic angiogenesis will improve clinical outcome following TBI.**

SPECIFIC AIM #1 Characterize/compare angiodynamics, neuronal injury, and cognitive deficits following TBI. Rationale: One of the characteristic pathologies of concussive type injury is development of DAI (89). While a number of laboratories have established a link between neuronal damage and cognitive outcome following injury (89-92), the association of cognitive deficit with injury in the Modified Marmarou model is defined. Further, it has not been determined whether the extent of neuronal injury correlates directly with loss of vascular function or inversely to the degree of vascular remodeling (angiogenesis). Using cortical impact or contusion models other labs have shown that to some degree angiogenesis is induced following TBI. However these studies have not fully characterized angiodynamics. In this proposal we will characterize endogenous mechanisms leading to increased capillary density in a closed-head injury model of TBI. We will distinguish newly formed from existing vessels as well as the kinetic appearance of signaling molecules involved in induction, pericyte migration, sprout formation and maturation of new vessels following TBI. We will focus on CNS centers (smCx and hipp) with known motoric and cognitive functions. Angiogenesis will be correlated to DAI.

Experiment 1.1. What is the extent of neuronal damage (DAI) in different sections of brain and cognitive impairment following TBI? Control (sham injured) animals will be transcardially perfused at 24 hrs and 48h post sham operation while injured animals will be perfused at 15 min, 30 min, 1h, 2hr, 4h, 12h, 24h, 48hr, and 7 days after injury (N=4 per group) for a total of 8 subgroups of animals. Following perfusion, brains will be removed and cryoprotected with 30% sucrose for 3 days. Brains will then be frozen on dry ice in mounting media and cryostat sectioned at 40μm sections throughout the area corresponding to smCx and hipp (between -1.8mm and -4.3mm from Bregma). Randomized sections throughout this region will be selected for analysis of DAI using immunocytochemistry for Beta-amyloid precursor protein (β-APP), nerve cell injury (Fluoro Jade), and cell death (TUNEL). These techniques have been successfully used to assess and quantify the extent of nerve cell injury/death for the last decade and are routinely used in our laboratory. Upon arrival animals will be acclimated to the housing conditions for a minimum of 24 hours. At this time baseline learning will be determined in eight-arm radial maze as detailed below. A second group of animals will be divided into two groups (N=6 per group). One group will receive sham operation, the other TBI. One hour after surgery animals will be tested for cognitive function using an eight-arm radial maze. Testing will continue until the animals have learned the task (defined as completion of the task ≤3 min) and remain at this level for 7 days.

Experiment-1.2. Evaluation of the post angiogenic response following TBI :temporal correlation with DAI. In these experiments we question whether there is a temporal correlation between the induction of angiogenesis and DAI following TBI and sham injured animals. Evidence of angiogenesis will be evaluated at various times (0-21days) following impact with and without normobaric hypoxia using the criteria listed in (**Table 1**): 1) increased capillary density; 2) expression of specific angiogenic proteins as detailed below; 3) presence of proliferating vascular endothelial cells. Control and injured animals will be used as detailed in Experiment-1.1, however animals will be sacrificed by decapitation. Tissue from smCx, corpus callosum, and hipp will be analyzed for protein express by Western analysis and immunocytochemistry. Expression of

specific mRNA transcripts will be quantified by RT- PCR. Sections from Protocol 1-1 will be analyzed with double or triple immunofluorescence for markers detailed in **Table 1** and GSA lectin (an EC marker), glut-1 (endothelial markers), NG2, PDGFβR or nestin (pericyte markers) and VEGF and VEGFR2. Immunocytochemistry using diaminobenzidine (DAB) immunoreactivity for GSA lectin binding or expression of glut-1 will be used to determine changes in capillary density. Further, in order to distinguish newly proliferating vessels, one more set of animals will be given a series of 5-Bromo-2'-deoxyuridine (BrdU) intraperitoneal (IP) injections (60mg/kg) 1,3,5,and 7 days prior to TBI. Sections will be taken as above and analyzed for BrdU using immunocytochemistry. Freshly isolated capillaries will also be examined for expression of angiogenic markers (**Table 1**). Newly formed vessels will also be characterized by the expression of MECA-32 a developmental endothelial marker upregulated at the onset of angiogenesis (93).

Table 1. Criteria for Angiogenesis

1. **Evidence of increased capillary density.**
2. **Upregulation of oxygen sensitive genes and genes involved in the angiogenic response: HIF-1α, HIF-2β, heme-oxygenase, increased glut-1, VEGF, EPO, increased Ang2,** p21*waf1/cip1* **and VEGFR2.**
3. **Evidence of proliferating endothelial cells (BRDU+) or Ki-67+ cells that express MECA-32.**

Interpretation and Limitations for Aim #1. Based on our own preliminary findings and those of other investigators, we predict that angiogenic factors will be induced following TBI beginning within 24 hrs following injury. We have chosen to do a comprehensive assessment of angiogenesis following TBI to gain further insight into the location and temporal appearance of angiogenic factors and whether there is an inverse correlation between DAI and evidence of angiogenesis. We predict that increased capillary density will occur following TBI (84). As in our previous published studies we expect this increase to be modest. It is unclear whether temporal differences in density will correlate with cognitive assessment. Another caveat is that we may not be able to find a link between increased capillary density in smCx, corpus callosum and hipp. We have determined that there may be a finite amount of new capillaries possible within the tightly compacted smCx and hipp. However, changes in surrounding tissue may improve blood flow and functional aspects of the tissue in these areas. Regional differences in capillary circulation are thought to underlie a number of pathophysiologic parameters seen in a large number of neurological diseases (83). Regional responses to hypoxia were observed in the *EMH-CreER^{T2}:ROSA26* mice (preliminary data).

As the techniques utilized for this aim are well validated we do not anticipate major problems. Further, as preliminary data has already shown evidence of neuronal damage, cognitive deficit, and angiogenesis, we predict that this aim will be straightforward. One potential pitfall is the number of animals used for Experiment 1.1 to establish the time course. It should be noted, however, that the same time course will be used in subsequent studies which will limit the number of groups needed. Further, the tissue obtained from these animals will be used in subsequent experiments. One potential caveat to the cognitive assessment is that neurological damage leading to motoric impairments as a result of injury could confound our data. However, in our experience less than 5% of the animals show any signs of neurological motoric deficit. Nonetheless, directly following TBI, animals will be screened for behavioral deficits using standard motor assessment paradigms (rotor rod, balance beam, and ladder climb). Any animal showing significant motoric deficit (see Methodology) will be excluded as this would likely decrease performance in the radial arm maze.

SPECIFIC AIM 2. Characterize angiodynamics following exposure to normobaric hypoxia.

Rationale: Transient or prolonged, acute hypoxia is a component of traumatic brain injury (44). In response to hypoxic stress the adult brain must undergo a number of adaptive processes that promote cell survival and maintain the balance between capillary density and neuronal metabolic demand. These include both systemic cardiovascular and respiratory adaptations (11) as well as acute adaptations that are organ specific and center at the microvascular level (10). Adaptive processes are intimately tied to unique features of cerebrovasculature and metabolic physiology and are likely to differ according to the duration of exposure to low O2 or specific cell type involved (10,11). Prolonged stress triggers a number of structural changes that restore baseline oxygen partial pressure. These changes involve increased capillary density with a resultant decrease in capillary distance. In the rat/mouse brain chronic mild hypobaric hypoxia induces a near doubling of the capillary density and produces an average intercapillary distance of 40 μm (10,38). **Increased capillary density coupled with decreased intercapillary distance results in a decreased diffusion distance that helps to restore tissue oxygen levels and promote wound repair. These adaptive changes are responsible for maintaining the balance between oxygen availability and tissue metabolic demand. In the brain there**

is a direct relationship between local capillary density, cerebral blood flow and neuronal metabolic demand. If maintenance of capillary density in the adult is ongoing and tightly regulated then dysregulation resulting in the loss of vascular and tissue homeostasis may lead to further adaptive responses such as astrogliosis (glutamate induced astrocytic glycolysis provides energy) seen in a number of pathological conditions. Loss of bioenergetic homeostasis is associated with neuronal injury/loss such as that seen following TBI.

We have extensive experience with three models of physiological angiogenesis (in vivo exposure to hypobaric hypoxia, in vivo exposure to normobaric hypoxia and in vitro hypoxia driven pericyte induced angiogenesis). We have chosen chronic mild normobaric hypoxia in order to eliminate possible effects of pressure in our system. [A detailed rationale for the use of therapeutic hypoxia can be found in the Background section under "Hypoxia the good bad and the ugly"]. In Aim 1 we will have determined the kinetics of normobaric hypoxia induced changes in angiodynamics. In this Aim we will utilize the following groups of animals: TBI impacted animals, sham-impacted animals and healthy mice. The same criteria of inclusion into the study as detailed in Aim #1 will be utilized in this Aim. Therapeutic angiogenesis through exercise can improve cognitive outcome following stroke presumably through restoration of normal blood flow to the brain (81,94,95). Exposure to normobaric hypoxia has been shown to result in an angiogenic response in the brain (10,11,38). While our laboratory and that of Joseph LaManna have considerable experience using models of physiological adaptive angiogenesis, most studies have been performed in the rat using hypobaric hypoxia (10,25). In our hands, the angiogenic response in the mouse following exposure to mild normobaric hypoxia is comparable to that observed following hypobaric hypoxia. However, for this study we feel that a careful analysis in mice to determine optimal conditions is warranted.

Experiment 2.1. Characterization of the angiogenic response following exposure to mild normobaric hypoxia using graded exposure to hypoxia. In this experiment we will determine the optimum conditions that result in increased vascular density (as a bio-indicator of adaptation to the hypoxic stimulus) in normal mice. While we have performed and published information pertaining to this question, in these experiments the location and temporal criteria will also be stressed. We will test graded exposure to normobaric hypoxia. Animals will be exposed to concentrations of oxygen from atmospheric 16, 14, 10 and 8%. Animals will be sacrificed after various duration of exposure from 0 to 21 days. Brain samples will be collected as detailed in Experiment 1.2 using the criteria for angiogenics outlined in **Table 1**. Vascular density will be determined using a stereological approach. Animals will be weighed and a blood sample will be taken prior to sacrifice to monitor hematocrit. The results will be a basis for subsequent experimental design.

Interpretation and Limitations. This experiment is geared to optimize the protocol to be used in the experiments detailed below. From preliminary results and our experience with the hypobaric hypoxia model, we expect that normobaric hypoxia at levels from 8-21% will have no adverse effects on control animals. At 8 and 10% oxygen adaptive angiogenesis is induced. Preliminary results suggest that aspects of physiological angiogenesis induced following normobaric hypoxia are similar to the in vivo system characterized by Joe LaManna and discussed in a number of excellent reviews (too numerous to cite). From results in preliminary data and in a published paper (11,93) we also know that mice respond similarly to rats. This is important, as experiments detailed in aim #4 will utilize a transgenic model generated on the C57BL/6J background.

Experiment 2.2. Does exposure to normobaric hypoxia following TBI lead to increased angiogenesis? Animals will be impacted and placed in normobaric hypoxia chambers at 72 hours post impact. We have previously determined that cerebral blood flow is normalized at this time. Chambers will be regulated exposed animals to a graded step down in oxygen concentration from 21% to 8-10%. Animals will be exposed to gradual decreases (2 degrees every 24 hours until concentrations reach 8% to 14% depending on the results in previous experiments. Tissue will be harvested after 24 hours, 2 days, 4 days, 7 days, 14 days and 21 days after TBI. The animals will be transcardially perfused and tissue will be analyzed as above for expression of angiogenic markers and capillary density (as detailed above in Aim#1). Animals will be sacrificed by decapitation (in a separate room from the housing room) and samples prepared as detailed above. Angiodynamics will be evaluated according to the criteria in **Table 1**. These results will be compared to those from TBI only animals and with those from sham-operated and non-exercised animals. **Expected results and limitations:** We have observed that there is an angiogenic response in areas of the brain associated with motoric and behavioral functions (13,14). Angiogenesis is induced following learning (34,35), exercise (12-14) and following chronic exposure to mild hypoxia (9,29). Recent studies from our laboratories have shown that

exercise conditioning leads to enhanced angiogenesis, increased capillary density, and decreased neuronal death (12-14,81,85). We have also shown in rats that exercise conditioning enhanced cognitive outcome in a model of stroke (13,14). It is likely that the same results will be seen following TBI. To be certain that interpretation of results will not be effected by confounding clinical conditions such as compromised cerebral blood flow we will initiate the exercise protocol only after CBF has returned to normal levels. This has been shown to occur by 3 days. From preliminary data we know that exposure to normobaric hypoxia induces physiological angiogenesis in control animals. Normobaric hypoxia also enhances the angiogenic response following TBI. Of interest, a comparison of exercise conditioning to normobaric hypoxia indicates that angiogenesis is greater and increased vascularity is more sustained following exposure to hypoxia than with exercise conditioning. We have shown that exercise conditioning enhances the angiogenic response following TBI. We therefore predict that normobaric hypoxia will also attenuate the angiogenic response following TBI in a more sustained manner.

Experiment 2.3 Determine the effect of normobaric hypoxia on neuropathologic/cognitive outcome following TBI. Rationale: Several reports have shown that angiogenesis is induced following TBI as well as other brain insults (81,85). We have shown that regulation of angiodynamics may be abnormal post TBI (14,23). This suggests that while there is an adaptive angiogenic response following TBI it may not be sufficient to compensate for the metabolic needs of the injured brain and thus may not be sufficient to buffer neurons from secondary damage. The inability to restore bioenergetic homeostasis and oxygen availability results in downstream hypoxic events, loss of tissue homeostasis and ultimately neuronal cell death. Such a mechanism may underlie the trauma induced cognitive impairment that has been observed even under conditions of mild injury in both animal models (89-92) and in human TBI (4-8). We have recently published that behavioral preconditioning prior to TBI augments adaptive angiogenesis and cognitive outcome (14). These findings support similar results reported in ischemic brain disease (15,96) and in nutritionally supplemented aged animals (14). We believe that adaptive angiogenesis is neuroprotective and that augmentation of angiogenesis underlies the cognitive sparing seen in our previous studies (14). **We hypothesize that post-traumatic treatment approaches that augment endogenous adaptive angiogenesis will have a profound effect on clinical outcome, will inhibit neuronal degeneration and will improve cognitive deficits.**

Experiment-2.3a: Does normobaric hypoxia improve neuropathologic outcome following injury? The same tissue from Experiments-2.1, 2.2 and 2.3 (TBI + will be analyzed for neuronal damage via the same assays in Protocol 1-1 (amyloid precursor protein [βAPP], TUNEL, FluoroJade). Tissue will be harvested at various time following exposure to normobaric hypoxia as outlined in experiment 2.2. Results will be compared with those of Aim 1 in sham operated and impacted animals to determine the extent of neuroprotection following injury. DAI will be quantified as detailed in methods below.

Experiment-2.3b: Does normobaric hypoxia improve cognitive outcome following injury? A separate group of animals will be subjected to TBI or sham injury with and without hypoxia. Animals will be tested on the radial arm maze after achieving the baseline state of performance (as detailed below). To confirm our results, as an alternate behavioral test, we will also use the Morris Water Maze. Results will be compared to those in Aim #1, Experiment-1.3 to determine whether normobaric hypoxia improves cognitive outcome following injury. Tests will be performed under the direction of Dr. Christian Kreipke who has considerable experience administering these tests. **Expected results and limitations:** Results from our laboratory indicate that induction of therapeutic angiogenesis through exercise conditioning improves cognitive outcome following TBI. We have not tested normobaric hypoxia. However, a search of the literature suggests that this may also be beneficial. The effect of exposure to hypoxia depends on the rapidity of induction, the duration of exposure and the level of hypoxia (as discussed in detail in section B). In general, exposure to low oxygen is well tolerated if there is acclimatization. Short acclimation with intermittent exposure to severe hypoxia can also trigger efficient physiological adaptation mechanisms. However, there is little knowledge about the cognitive repercussions. In one study, Leifflen and colleagues (97) showed that short acclimation protocols based on intermittent exposure to simulated high altitudes triggered adaptive processes without major impairment in a choice reaction time task during acute stages of severe hypoxia. Another recent study has shown that acclimatization to high altitude prevents the impairment of classical eye blink conditioning evoked by hypoxic conditions but does not improve this task when acquired under land conditions (98). Of note is that these authors exposed animals only for one week. At this time there is as yet no structural adaptation to low oxygen.

Another study (99) examined the effect of intermittent hypoxia on cognition in adult mice. Mice were exposed to 2, 5, 10, 15, or 25 days of intermittent hypoxia (4 hr/day) at 2 km (16.0% O2) or 5 km (10.8% O2) altitudes. Results in the Morris Water Maze task showed that exposure to hypoxia at either concentration did not change the escape latencies of mice in the training test or the retention of platform in the probe test. In the shuttle-box task, however, hypoxia reduced mouse avoidances in the acquisition test and reduced avoidances in the retention test at some time points. While none of these studies recapitulates the protocols in this study, they do suggest that hypoxia in adults may impair hippocampal-independent, but not the hippocampal-dependent, tasks in mice. Of note is that 4 hours per day may be insufficient to induce proper adaptation. In one other study, Sen Gupta (100) reported that endurance physical training at moderate altitude improved hypoxic tolerance and the central nervous system activity. In all other studies, investigators simulated acute severe hypoxia for short periods of time inducing varying degrees of CNS damage. No damage should be seen following graded exposure to chronic mild levels of hypoxia. We therefore expect to see an improvement in cognitive performance.

Experiment-2.4. How long after injury can therapy be initiated with therapeutic efficacy? In a separate set of experiments animals will be subjected to injury or sham injury. Normobaric hypoxia will be initiated (using the optimal conditions established in previous experiments) at various times following impact (immediately following recovery, 72 hours, 1 week,2 weeks and 3 weeks). It is assumed that in combat situations it may not always be possible to initiate therapy. Soldiers may need to be transferred to convalescent facilities to begin therapy. Therefore we question, in this experiment, whether induction of physiological angiogenesis even as long as three weeks following injury will be beneficial. **Expected results and Limitations.** Based on our previously published and preliminary data we anticipate that normobaric hypoxia will induce angiogenesis. Further, we predict that exposure to normobaric hypoxia will induce maximum and more sustained increases in capillary density. Angiogenesis is predicted to increase in parallel with a decreased neuropathology, as the neuroprotective role of new vessel formation and maintenance of bioenergetic homeostasis has been well established (75). These improvements will likely lead to improved cognitive outcome in the radial arm maze. Further, it is predicted that we can further improve cognition following injury with exposure to normobaric hypoxia. While there are a number of other techniques that might be used to induce adaptive angiogenesis (VEGF injections for example) we have chosen to use normobaric hypoxia because of the ease of administration, the relative lack of expense, and that the increase in capillary density induced by this technique is physiological and has been shown to promote survival.

One problem, as discussed above, is the neurological status of the animals following injury. However, as previously described, we will overcome this by testing each animal for motoric function. Any animal performing at an impaired level will be removed from the study, as performance on the radial arm maze would be hampered by impaired ability to move about the apparatus. HOWEVER, it is important to note that animals that have altered neurological parameters may benefit from normobaric hypoxia where performance on the radial maze is not a factor. For that reason we will consider studying these animals using normobaric hypoxia although the Interpretation of the data may be difficult.

Specific Aim #3. To confirm the mechanistic underpinnings of neuroprotection and augmentation of angiodynamics produced by normobaric hypoxia in TBI. Rationale: In this proposal we consider a treatment paradigm that promotes endogenous adaptive mechanisms that improve vascular function and bioenergetic homeostasis. In the treatment paradigm an angiogenic response is induced by chronic exposure to mild hypoxia. Physiological or adaptive angiogenesis is a complex stress response. It induces a number of alterations in the basic physiology of numerous processes. Within the framework of this study it will not be possible to test all possible mechanisms. **We will question whether pharmacologically induced or inhibited angiogenesis supports the concept that physiological angiogenesis is protective in TBI. We will also focus on the role of HIF-1α stabilization.** The experiments in Aim #3 are designed to question the potential mechanisms, and in so doing, pinpoint potential pharmacological agents that might substitute or synergize with therapeutic angiogenesis.

Question #1: Can pharmacologic modulation alter therapeutic angiogenesis? Rationale: These studies are designed to provide key information about the mechanism underlying the beneficial effects of angiogenesis on both neuropathologic and cognitive outcomes following injury. To confirm whether any effects of normobaric hypoxia that may exist are due to angiogenesis, we will block angiogenesis via multiple mechanisms during exposure to normobaric hypoxia following TBI and test the same outcome measures as

above. In doing so, we will shed light onto the contribution of angiogenesis to the positive effects of normobaric hypoxia to outcome following TBI. As further evidence of its role in mediating positive outcome, we will then pharmacologically induce angiogenesis following TBI and compare these results to those obtained during normobaric hypoxia.

Experiment 3.1. Does pharmacologic inhibition of angiogenesis alter normobaric hypoxia induced neuroprotection? In these studies pharmacological inhibition of angiogenesis will be initiated at the onset of exposure to normobaric hypoxia following TBI. Vehicle alone will be used as control. Animals will be tested for cognitive changes as detailed above and sacrificed at varying periods of time to assess angiodynamics and capillary density using criteria detailed in **Table 1.** We will examine three inhibitors of angiogenesis targeting three different pathways (HIF-1 stabilization, downstream HIF-1 gene activity, HIF-1 transactivation). **Antimycin A1 (150ng/g/day to 300ng/g/day)** has been identified as an inhibitor of angiogenesis (101). Its mode of action is thought to be inhibition of HIF-1 transactivation and subsequent downregulation of VEGF. No effect is seen on transcriptional activity. **Endostatin (5mg/kg/day)** is a 20-kDa endogenous angiogenesis inhibitor that inhibits the expression of VEGF in a HIF-1 independent manner. **Histone deacetylase inhibitors** (HDACIs) are currently in clinical trials due to their potent anti-angiogenic effects. HDACIs (phenylbutyrate, sodium butyrate, TSA, SB, Apicidin, and VPA) dramatically decreased HIF-1alpha protein level and transcriptional activity of HIF-1 in human and mouse tumor cell lines. Conversely, class I HDACs enhance HIF-1alpha stability and HIF-1 transactivation function in hypoxic conditions. We will use the FDA approved drug **phenylbutyrate (PB) (1mM/kg/day oral gavage**) in these studies. Changes in capillary and whole brain HIF-1 expression will be determined at the transcriptional and protein level. **Expected results and limitations:** We expect that inhibition of angiogenesis will inhibit the beneficial effects of normobaric hypoxia-induced amelioration of cognitive decline following TBI. The anti-angiogenic effects of antimycin and endostatin are well known. The doses proposed are well below any toxic levels. We have extensive experience with PB and with oral gavage techniques. Anticancer effects of PB may be through inhibition of angiogenesis (102-104). PB is safe and has no known side effects. However, it is unclear whether PB alters HIF-1.

Experiment 3.2. Does pharmacologically-induced angiogenesis recapitulate neuroprotection induced by normobaric hypoxia augmented adaptive angiogenesis? In this experiment we will question whether pharmacologic induction of angiogenesis recapitulates the effect of adaptive angiogenesis following TBI. Inhibitors of prolyl hydroxylase have been tested for their ability to induce HIF-1α stabilization and subsequent expression of target genes (105,106). Among them, 3,4-dihydroxybenzoic acid, deferoxamine (DFO), and DP have neuroprotective effects against brain ischemia (106-110). It is unclear whether these agents act solely on the HIF pathway. Baranova and colleagues (107) found that administration of these compounds led to significant accumulation of HIF-1α protein in the cerebral cortex at 6 h after drug delivery accompanied by a significant upregulation of VEGF and Glut-1 mRNA levels at 24 h after administration indicating a functional activation of HIF-1α. In our studies two approaches will be taken: A) Administration of a low molecular weight inhibitor of HIF prolyl 4-hydroxylase (DFO) and 3,4 DHB available from Sigma). B) Administration of an angiotensin II receptor blocker (ARB available from Novartis).

Experiment 3.2A. To determine whether prolyl hydroxylase inhibition with HIF-1 stabilization is neuroprotective in TBI. We will treat animals with DFO, 3,4-DHB. DFO, and 3,4-DHB. These agents were selected for testing because their mechanisms of prolyl hydroxylase inhibition are believed to be distinct. 3,4-DHB has been shown previously to be an inhibitor of collagen prolyl 4-hydroxylases with respect to the 2-oxoglutarate and ascorbate cofactors and to be neutral with respect to Fe2+ (111). By contrast, the ability of DFO to inhibit recombinant prolyl 4-hydroxylase activity can be overcome by addition of exogenous Fe. Animals will be injected IP with 3,4-DHB (160mg/kg/day). Drug will be given at various times following TBI. Samples will be taken as detailed in aim #2 to document angiodynamics. Animals will be tested for motor, memory as detailed in Aim #2. Westerns will be performed using a specific antibody to HIF-1α, HIF-1β, HIF-2 as described above. All of these agents (100 μM DFO, 10 μM dihydroxybenzoic acid) stabilize HIF-1 under normoxic conditions. This experiment will determine whether stabilization of HIF-1 alone is sufficient to be protective in TBI.

Experiment 3.2B. to determine whether pharmacologically increased capillary density recapitulates adaptive angiogenesis in TBI. Animals will be injected with the angiotensin II receptor1 blocker (ARB) (Novartis Pharma AG) valsartan. Valsartan as well as other ARBs have been found to increase capillary density. Valsartan will be infused at 2-3mg/kg/day. This dosage does not induce hypotension (112). Animals

will be impacted than exposed to exercise or normobaric hypoxia with or without valsartan. Angiodynamics will be assayed as in **Table 1**, and DAI assayed as detailed above. This experiment will tell us whether the actual increase in capillary density is sufficient for neuroprotection. **Expected results and limitations:** It is unclear whether neuroprotective mechanisms that ameliorate cognitive decline and neuronal injury require the structural increase in vascularity or whether the effect is mediated by the induction of a gene or genes induced during enhanced angiodynamics. Within the framework of this study the proposed experiments pinpoint possible mechanisms in order to provide rational for the design of potential adjunct therapy that could augment endogenous angiogenesis. We have targeted HIF-1 and VEGF and have proposed to use a drug that enhances vascularity in normal animals. We expect that this drug valsartan will augment both exercise and normobaric hypoxia-induced angiogenesis. It is unclear what the potential mechanism might be. However we propose that pharmacologic intervention with agents that induce an increase in vascular density may be used in patients that are unable to begin exercise or be transferred to a hypoxia chamber or to high altitude. That the HIF-1 response appears to suboptimal in capillaries isolated from animals exposed to TBI suggests that the increase observed following hypoxia may be due to induction and stabilization of HIF-1α.

Question #2 Therapeutic Angiogenesis stabilizes the HIF-1 response. Rationale: Cells exposed to low oxygen concentrations respond by initiating defense mechanisms, including the stabilization of hypoxia-inducible factor (HIF)-1α, a transcription factor that upregulates oxygen responsive genes such as those involved in glycolysis and angiogenesis. It is thought that mitochondria regulate the stability of HIF-1α through the increased production of reactive oxygen species (ROS) (87-89). Nitric oxide and other inhibitors of mitochondrial respiration prevent the stabilization of HIF-1α during severe hypoxia due to an increase in prolyl hydroxylase–dependent degradation of HIF-1α (88). We have preliminary data indicating that capillary pericyte HIF-1α is altered following traumatic brain injury. This suggests that pericyte mitochondrial oxygen sensing mechanisms may be compromised following TBI. We have previously published that moderate TBI results in vascular dysfunction. Pericytes migrate from their capillary location and non-migrating pericytes undergo apoptosis (23). There is endothelial activation (25) and the pericyte VEGF angiogenic response is altered (18). As pericytes have an important role in the induction of angiogenesis, compromised pericyte function could blunt the adaptive processes required to maintain bioenergetic homeostasis in the traumatized brain. **We hypothesize that normobaric hypoxia shifts the pro-apoptotic HIF-1 response to a pro-survival response increasing vascular density. Chronic mild hypoxia stabilizes HIF-1α, and upon induction of angiogenesis, results in new vessel formation associated with pericyte proliferation and restoration of vascular function.** The experiments in Aim #3 are designed to question the potential mechanisms, and in so doing, pinpoint potential pharmacological agents that might substitute or synergize with therapeutic angiogenesis.

Experiment 3.3 HIF-1α accumulation following TBI: the effect of normobaric hypoxia. We will utilize the following groups of animals: TBI impacted animals, and sham impacted animals. The same criteria of inclusion into the study as detailed in Aim #1 will be utilized. Mice will be impacted and exposed to normobaric hypoxia as detailed above. At 0, 1, 2, 3, 4, 7, 10, 14 and 21 days animals will be sacrificed by decapitation and samples prepared as detailed above to establish evidence of induction of angiogenesis. The same criteria for angiogenesis detailed in Aim #1 will be used. Capillary and whole brain expression of HIF-1α, HIF-1β and HIF-2α mRNA will be quantified by qRT-PCR, and brain, capillary and nuclear protein by western analysis. HIF-1α localization to pericytes can be determined by in situ hybridization and by dual staining to detect co-expression of pericyte NG2/HIF-1α. **Expected results and limitations:** From preliminary results and from experiments performed above we know that the HIF response following TBI appears to be altered following TBI. Results show that HIF-1α protein levels are increased but the level of increase is much lower than that observed following exposure to mild hypoxia. Message is induced at a much later time period and was only observed when we examined capillary RNA. This may suggest that HIF-1α is not produced by parenchymal cells such as astrocytes shown previously to express HIF-regulated genes under hypoxic conditions. This should be apparent in stained sections. While an increase in message is not observed in all cell systems following a hypoxic stimulus, in the brain, both responses are observed. In capillaries we expect to see that the earliest HIF-1α will co-localize with pericytes confirming our results in hypoxia. As pericytes migrate within 24 hours post impact these events may be difficult to see. It is possible however to clearly delineate nuclear HIF-1α in capillaries and nuclear isolates. Pericytes have round nuclei and endothelial cells have elongated nuclei. HIF-1

responsive gene products can also be visualized in capillaries using dual or triple label immunofluorescence techniques.

Experiment 3.4. HIF-1α gene activity. To verify increased stability of HIF-1α we will question whether there is associated upregulation of HIF-1α dependent oxygen sensitive proteins. We will examine samples from animals exposed to TBI with and without exercise conditioning and/or normobaric hypoxia for the expression of the glucose transporter protein (glut-1), VEGF, erythropoietin (EPO), and p21*waf1/cip1*, known HIF-1α-regulated genes. Beta-Tubulin and angiopoietin-2 will be used as a control as their transcriptional regulation is HIF-independent. Samples of whole brain and isolated capillaries will be harvested from animals using the protocol in Aim #2. Gene activity will be analyzed by RT-PCR and protein by western analysis. **Expected results and limitations:** While these experiments are correlative they provide a basis to further assess the angiogenic response in TBI. Our selection of HIF-responsive genes should pinpoint compromised oxygen and glucose delivery (glut-1), neuroprotection (EPO) as well as altered regulation of angiogenesis (p21cip1). We have previously published that pericytes synthesize and release the eicosanoid PGJ2 within 15 minutes in response to hypoxia and that this involves cMyc. There is cell cycle arrest and subsequent migration of pericytes. Migration promotes survival and suggests that HIF-1α-Myc may be involved (113). An in depth study of the HIF-1/c-Myc adaptation is beyond the scope of this in vivo study. However, results may yield clues that may suggest compromised HIF-1α regulation of c-Myc stop and go signaling. This will be addressed in future studies if results point to this possibility.

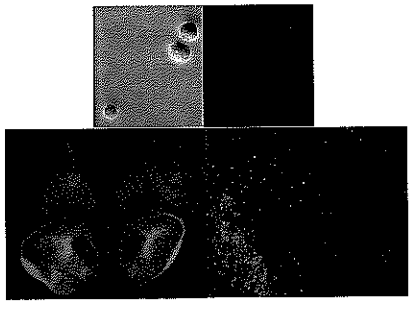
Experiment 3.5. HIF Prolyl hydroxylase in TBI, with and without normobaric hypoxia. In normoxia HIF-1α is targeted for hydroxylation by prolyl hydroxylase that signals recognition by a ubiquitin ligase complex containing the von Hippel-Lindau tumor suppressor. Three prolyl hydroxylases have been identified (EGLN1, EGLN2 and EGLN3). Accumulation of EGLN1 due to hypoxia induced increases at the mRNA level inhibits HIF-1α transcriptional activity and thus functions as a feedback inhibitor. We question whether prolyl hydroxylase activity or regulation is altered following TBI and whether the beneficial impact of exercise conditioning and/or normobaric hypoxia is related to inhibition of prolyl hydroxylase. Prolyl hydroxylase activity will be determined by an in vitro hydroxylation assay using capillary lysates (114). Capillaries will be isolated at 8 and 24 hours following TBI, TBI plus exercise conditioning and/or normobaric hypoxia. Prolyl hydroxylase protein levels will also be determined. **Expected results and limitations**: Although prolyl hydroxylation is a critical step during HIF-1α regulation, there is currently no convenient way to measure activity. Standard in vitro biochemical assays measure the conversion of radiolabeled α-ketoglutarate to CO_2. Although quantitative, this method is limited to in vitro studies and often encounters high background signals due to uncoupled reactions. It also requires special equipment to handle radioactive gas. Another widely used method to measure HIF-1α prolyl hydroxylation is the pVHL capture assay. However, this technique involves multiple steps such as immunoprecipitation or far-Western assays. The most convenient way of measuring HIF prolyl hydroxylation is to use a hydroxyproline-specific HIF-1α antibody. Unfortunately, none of these methods allows the simultaneous detection of both hydroxylated and nonhydroxylated HIF-α, making them less amenable to quantification. An alternative approach will be to construct a fusion protein containing HIF-1α, which exhibits differential migration upon SDS-PAGE when hydroxylated. This approach was successfully used by Pan and colleagues (114) for both in vitro and in vivo studies. The worst-case scenario is that only hydroxylase transcript and protein levels will be measured.

Experiment 3.6. In vivo Inhibition of HIF-1α with small interfering RNA (siRNA): HIF-1α may have both positive and negative effects on disease pathology. In severe hypoxic conditions HIF-1α may induce apoptosis. Inhibition of HIF-1α by siRNA has been shown to improve clinical outcome in ischemia reperfusion injury when added early (115). SiRNA can be used in vivo by direct injection using stereotactic means. Dr Vladimir Katyshev in my laboratory has been trained in stereotaxy instrument guidance techniques while he was in Russia. In tissue culture use of siRNA is relatively easy and the use of HIF-1α siRNA inhibits 75% of pericyte hypoxia induced HIF-1α protein and 80% of HIF -1α dependent gene VEGF (data not shown). In our initial in vitro experiments siRNAs for HIF-1α were designed by searching the coding sequence for two adenines followed by 19 nucleotides that had a GC content that was below 45% and did not contain more than three thymines or adenines in a row. These sequences were tested for possible homology to other mouse genes with BLAST. Four potential siRNA sequences were selected and prepared using Ambion (Austin, TX) silencer siRNA construction kit. After screening, siRNA 1589 (UUCAAGUUGGAAUUGGUAG) was selected for further use. We used a commercial Santa Cruz HIF-1α sc35562 to validate the siRNA. A negative control was

designed by randomizing the sequence (AAUUAGCGUAGAUGUAAUGUG) and checking for nonhomology by BLAST. The Santa Cruz siRNA is composed of pools of 3-5 target specific 19-25 nt siRNAs designed to knockdown gene expression. We will test the siRNA following in vivo administration. If our hypothesis is correct then HIF-1α siRNA injected following impact should inhibit the therapeutic effect of normobaric hypoxia. Transfection will be confirmed by use of fluorescent RNA, protein by western analysis and transcript by RT-PCR. **Expected results and limitations:** We expect that injection of siRNA into the CNS will be effective in that the brain is protease free. It is likely that inhibition of HIF-1α by siRNA will affect multiple cells and many areas of the brain. While it is clear that pericytes mount a Hif-1α response it is also possible that other cells such as neurons or astrocytes similarly respond (10,11). If our hypothesis is correct, inhibition of CNS HIF-1α and thus HIF stabilization will eliminate the therapeutic potential of normobaric hypoxia.

Experiment 3.7. Fate mapping the cell specificity of the biphasic HIF-1α response in TBI and/or normobaric hypoxia in transgenic mice. We generated transgenic mice that harbor a tamoxifen-responsive Cre-recombinase ($CreER^{T2}$) (116) under regulatory control of a hypoxia sensitive promoter/enhancer [*EMH-CreER^{T2}:ROSA26* mice]. This transgene is activated in cells that mount a HIF-1α response and the *lacZ* reporter gene is expressed. The time of reporter activation can be varied by dosing the mice with tamoxifen at varying times post impact. These animals will be used to Fate Map the HIF-1 responding cell at various times post TBI with and without normobaric hypoxia. Littermates with the Cre(α):HIF-1αF/F genotypes, transgenics without tamoxifen and wild-type C57BL6/J mice (Wt) will be used as controls. Histology and characterization of the angiogenic response will be determined as discussed above. Expression of protective (EPO, glut-1, VEGF, p21cip1) and pro-apoptotic (bNIP-3, RTP801) HIF-1α regulated genes will be examined by western blotting, RT-PCR at the whole brain and capillary level. Immunohistochemical or FISH analysis of capillaries will also be performed. Experiments will compare wild type mice, our tamoxifen-regulated *EMH-CreER^{T2}:ROSA26-lacZ* mice and mice lacking the transgene. **Expected results and limitations:** In these experiments transgenic animals can be used to pinpoint specific cellular HIF-1α responses in TBI with or without normobaric hypoxia. Our results discussed above suggest that the HIF-1α response is first seen in pericytes within 24 hours of induction of adaptive angiogenesis. In TBI, pericytes either migrate or become apoptotic by 24 hours. Baronova and colleagues have shown that in stroke models there is a biphasic HIF-1α response (107). The first response was seen within 24 hours and was associated with induction of pro-apoptotic genes bNIP-3, RTP801, Noxa, and Nix. These investigators did not determine the cellular localization of these molecules. That HIF-1α may be involved in induction of pro-apoptotic molecules fits with our observation that pericytes remaining in their capillary location following TBI undergo apoptosis whereas those that migrate survive. The results from these experiments should pinpoint the early and late cellular localization of these molecules. The second or late phase of the HIF response to injury occurs after a transient loss of HIF-1α stabilization resulting in a subsequent increase for 8 days. Loss of neuronal HIF-1α results in exacerbation of the injury in the MCAO model of stroke and is associated with decreased vascular density (107). We may see similar results in TBI. Our preliminary results suggest that the late HIF-1α phase is compromised following TBI. It is possible that DAI in these animals results in inhibition of neuronal HIF-1α. If results pinpoint a major role for the neuronal HIF-1 response, we will study a transgenic mouse produced by Dr. Caesar Chavez (107) in collaboration with Dr. Joseph LaManna. This animal is a conditional knockdown, in which HIF-1α expression is inhibited in neurons (107). This deletion was achieved by crossing mice bearing loxP-containing (floxed) HIF-1α alleles (HIF-1$\alpha^{F/F}$) with transgenic mice expressing Cre recombinase under the control of the CaMKIIα neuronal promoter (HIF-1$\alpha^{\Delta/\Delta}$).

Future studies: If studies continue to implicate pericyte dysfunction in TBI we will perform experiments to examine pericyte stem cell activity as well as perform experiments that involve i.v. injection of pericytes. We have reported that pericytes are a source of adult stem cells (papers appended). Purified pericytes can be injected i.v. [as little as 10^4 cells] and have been shown to migrate to all organs tested thus far (Dore-Duffy unpublished observations). However, migration is highest to injured tissue. For example pericytes (labeled with red tracking dye) migrate to the spinal column [shown in the figure to the right] in



experimental autoimmune encephalomyelitis (EAE) and have been found to ameliorate clinical symptoms in this murine model of human multiple sclerosis. The mechanisms appear to involve restoration of vascular homeostasis. Similar studies will be performed in TBI. Pericytes will be isolated from syngeneic brain tissue and 10^5 to 10^6 cells injected i.v at various times post impact. Angiodynamics will be evaluated as detailed above.

GENERAL METHODS

Closed Head Trauma Model: Adult male/ female C57BL/6 mice (Jackson Laboratories) will be anesthetized with 5% halothane in 2% oxygen prior to intubation, and then maintained on 1.5% halothane via a mask and spontaneous breathing. A mid-sagittal scalp incision will be performed and the underlying muscles retracted laterally. Cranioplastic cement will be used to attach a 10mm diameter X 3 mm thick, round metal helmet directly to the skull over the sagittal suture and between the coronal and lambdoidal sutures. The helmet is used to distribute the applied force over the surface of the parietal bones, preventing skull fractures and penetrating brain injury. After the cement dries the animals will be placed prone on a platform as described in the Acceleration Impact Trauma Model of Marmarou (78). After 30-40 seconds of placement, 450g of weight contained in a hollow plastic cylinder will be dropped directly onto the helmet from a height of 2 meters. Following a brief convulsion and respiratory arrest, most animals restart breathing on their own. However, in some cases, the use of a rodent respirator or CPR is necessary prior to spontaneous breathing. Using these precautions, mortality has been reduced to less than 5%. In some animals after impact, the helmet will be removed and the skin sutured only if the skull shows no evidence of fractures. After suturing the skin, sensory cutaneous and evoked motor responses will be tested. Usually the intubation tube is removed at 10 min post trauma and only animals that are able to right themselves before 30 min after injury will be included in the study. Brain and leg muscle temperatures are taken routinely up to 24 hrs post injury. Brain temperature fluctuates +1.5°C, and muscle temperature 1.3°C.

Mice were subjected to TBI using a standardized weight-drop device. In brief, after induction of anesthesia, the skull will be exposed by a midline longitudinal scalp incision. The head was fixed and a 250 g weight will be dropped on the skull from a height of 2 cm, resulting in a focal blunt injury. After trauma, the mice receive supporting oxygenation with 100% O_2 until fully awake. The extent of posttraumatic neurological impairment is assessed at defined time intervals after trauma using a standardized Neurological Severity Score (NSS). A 10-parameter score will be used for assessment of posttraumatic neurological impairment. The NSS will be assessed in a blinded fashion by two different investigators. The score comprises 10 individual parameters, including tasks on motor function, alertness, and physiological behavior, whereby one point is given for failure of the task, and no point for succeeding. A maximum NSS score of 10 points indicates severe neurological dysfunction, with failure of all tasks.

Exercise Conditioning and Radial Arm Maze Setup: Exercise conditioning will be performed in the same room that the animal resides. Following an initial acclimation to treadmill apparatus each day animals will be placed on the treadmill following a standard protocol developed by Musch and Terrell (83) in which exercise is initiated for 2 min with a warm-up period at a speed of 16 m/min up a 5% grade. Over the next minute treadmill speed and grade are progressively increased to a speed of 28 m/min at 20% grade. This routine is considered moderate activity with no stress to the animal (74). Animals will be held at this level until 30 min of total exercise is achieved. The non-exercised rodents will be put on the treadmill for the 30 min but the treadmill will not be turned on. Two independent investigators will monitor the time. Exercise conditioning will take place during the dark phase of the diurnal cycle so as to minimize effects of circadian rhythms. Cognitive testing will take place directly following 30 min of exercise conditioning.

From day 1 to day 3 of the cognitive study, animals will be handled by the researcher for 10 to 15 min each. To acclimate them to the maze, mice will be placed onto the central platform of the radial arm maze and allowed to roam freely. To provide sufficient motivation for foraging in the maze, animals will undergo a mild dietary restriction. Chow will be gradually decreased from 20mg (5 pellets) to a minimum of 18 mg. A custom designed radial arm maze has been built using black acrylic sheet (0.6 cm thick). Eight identical radial arms are fixed to an octagonal base platform that stands 63 cm above the floor. Each radial arm is 60 cm in length and 10 cm in width with 10 cm – high sidewalls along each arm. At the end of each arm a 5-cm end piece is placed. A hole measuring 2.5 cm in diameter is 5 cm from the end of each radial arm to place a plastic food cup (1 oz). During cognitive testing, the maze is enclosed within four black linen walls. A white paper triangle (15-cm sides) is placed on one linen wall 10 cm above the base of radial arm #3. An 8" x 11" white paper square with bisecting black lines is placed on the same linen wall 10 cm above the base of radial arm #5. A

researcher holding a green notebook and always wearing a bright yellow surgical gown with latex gloves will be positioned in front of radial arm #8 during the study. These three visual cues provide spatial guidance for the location of the baited arm containing the food. Radial arm maze trials will commence from the 3rd day following TBI. The time taken to find the bait (half of a Fruit Loop cereal®) placed in a plastic cup of four different radial arms will be tested daily for three consecutive time trials. Animals will be allowed to spend 10 min maximum in the maze. Averages of these trials will be calculated and recorded.

Assessment of motor deficit: In order to screen animals for motor deficit, all TBI animals will be tested using standard neurological function tests, including rotor rod performance, balance beam, and ladder climbing, all of which are currently routinely used in our laboratory. Since it is imperative that our latency data not be confounded with neurological problems, exclusion will be based on failure to accurately complete one of the three measurements. Based on preliminary screens, animals either perform well or show deficit on all tests. Animals performing at sub-control levels on any test will be removal from the study.

DAI assessment: Animals will be transcardially perfused with normal saline followed by 4% paraformaldehyde. Brains will be harvested and post fixed in the same perfusate for 24 hrs followed by cryoprotection with a solution containing 20% sucrose in 4% paraformaldehyde. Serial sections (40 μm) of brain tissue containing the smCx and hipp will be collected in multi-well plates and processed for beta amyloid precursor protein (β-APP) immunocytochemistry. Sections will be washed in PBS and treated in 80% formic acid for 10 min.washed and endogenous peroxidase activity blocked with 0.3% hydrogen peroxide in PBS for 1 hour then incubated overnight (1ug/ml) with a rabbit anti-APP C terminus antibody (Zymed Laboratories, CT 695). The antigen-antibody complex will be revealed using the avidin biotin peroxidase method using DAB as a substrate and viewed with a Leica light microscope fixed with Axiocam software. Images of labeled retraction bulbs from hipp and smCx will be collected and quantified. Manual counting of retraction bulbs and axon fragments in predetermined areas of white matter will be used to ultimately determine the extent of DAI.

FluoroJade: FluoroJade will be performed as previously described (69). Briefly 40 μm sections from smCx and hipp are washed in 80% ethanol with 1% NaOH, 70% ethanol and distilled water. Next tissue sections are washed in 0.06% $KmNO_4$ for 10 min and then washed in distilled water. FluoroJade solution (2 mL) is diluted in 48 mL 0.1% acetic acid and tissue is incubated for 30 min. Sections are then rinsed in distilled water, dehydrated, air dried and mounted with water-based mounting media.

TUNEL: TUNEL is performed on 40μm sections from smCx and hipp using a commercially available In Situ Cell Death Detection Kit (Boehringer Mannheim, Brisbane, QLD, Australia). Briefly, tissue is fixed as and mounted onto poly-l-lysine coated slides. Slides are soaked in xylene (twice for 5 min) then ethanol (3 min in 96, 90, and 80%) then rinsed in de-ionized water. Tissue is permeabilized with proteinase K 15 min at 37°C, rinsed in PBS and incubated 60 min in the TUNEL mixture. After incubation, sections are rinsed in PBS, treated with alkaline phosphatase. TUNEL-positive nuclei are counted.

Exposure to normobaric Hypoxia (In vivo model): Experiments will be performed on site. Animals will be kept one per cage for the indicated experimental periods in normobaric chambers (3 cages per chamber). Animals will have normal access to food and water. Cage cleaning, water, and food can be given or performed within the chambers using front armhole access. Littermates will be kept side-by-side chambers at normoxia. They will be otherwise treated exactly as the experimental group. Animals will be exposed to stepwise-graded concentrations of oxygen (atmospheric > 16 > 14 >12 > 10%). Concentrations will be changed every 24 hours. Animals will be exposed to continuous hypoxia for a minimum of 8 hours/day. This is a time we have determined to induce maximum changes in vascular density. Animals will be removed to normobaric normoxia to do motor testing in the water maze. Surgical Procedures: Surgical procedures on all mice will be performed under conditions of normobaric hypoxia or normobaric normoxia. On the day of the experimental procedure mice will be weighed and then decapitated within 5 min of removal from the hypobaric chambers. Alternatively animals will be perfused as detailed for specific experiments. A sample of blood will be obtained from the tail artery just prior to decapitation to measure hematocrit. The brain will be removed using sterile procedures and samples and/or microvessels will be isolated as described below.

Protein isolations. Cortical samples and isolated microvessel samples will be homogenized in ice-cold lysis buffer (1x PBS, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 100mM Na3VO4) or aprotinin, 100µg/ml PMSF, 1µg/ml pepstatin). Homogenates will be centrifuges at 20,000 g for 30 minutes 4° C and the supernatants used for western blot analysis. Protein content in the supernatant will be determined using a Bradford protein assay (Bio-Rad) with bovine serum albumin as a standard

Western Analysis: Whole brains will be harvested (n=3 animals per group), placed in cold isobutane and partially frozen. Brains will then be dissected to isolate smCx and hipp. Isolated smCx and hipp will be homogenized in Lamelli's solution and subjected to SDS-PAGE. Protein concentrations will be standardized for all samples. Controls are run in the same sets. Gel proteins are transferred to PVDF membranes and incubated with 1% skim milk + Tris-saline for overnight to block non-specific Ab binding. Membranes are incubated with antibody (predetermined to function in western analysis) at saturation density for 2-4 hours at room temperature, washed and incubated with tracer antibody. Nitrocellulose will be rinsed in Lumiglow solution for 90 seconds, exposed to X-ray film and developed. Band intensity of immunoblots will be quantified using densitometric analysis. We have used horseradish peroxidase conjugated anti-specific IgG but are also looking at other luminescent detector systems. Chemiluminesence will be evaluated using our Waters fluorescence detector. Radioactivity will be determined by autoradiography and densitometry.

Immunocytochemistry,Frozen Sections: Experimental animals will be perfused with saline and 4% paraformaldehyde and the brain will be immersed in the same fixative for 1-2 days. After fixation the brains will be dissected in 1-cm3 blocks, cyroprotected with sucrose, and frozen on dry ice. Sections, 6 µm thick, will be prepared with a cryostat (Carl Zeiss, Microm HM505N, Germany). The sections are stored at -20°C before use. Multiple series of coronal sections (40 μm thick) through the smCx and hipp will be cut on a cryostat and collected in 1% PBS as free-floating sections. The series consist of tissue from each group of animal and will be used for detecting the following: BrdU, VEGF, lectin+VEGFR2, and lectin alone. Tissue will first be permeabilized with 0.6% Triton X. The monoclonal antibody generated against BrdU is conjugated to FITC (1:150; Sigma, St. Louis, MO). The lectin (B*anderiaes simplicifolia*, 1:100; Sigma) is conjugated with FITC and was previously used by us to visualize endothelial membranes. VEGFR2 will be detected using a monoclonal antibody (1:100; Sigma). For visualization, antibodies are biotinylated using a Vectastain kit (1:100; Vector Laboratories, Burlingame, CA, USA) followed by Texas Red (TR) conjugated to streptavidin (1:100; Jackson Immuno Research Laboratories, Westgrove, PA, USA). Second antibody is used as a control.

Determination of Capillary Density: Four sections taken from different sections of the brain will be analyzed per animal. 40μm sections corresponding to smCx and hipp will be immunostained for ECspecific lectin coupled to FITC or specific antibody directed against the glucose transporter protein (glut-1). We have also used antibody directed toward PECAM. Anti-FITC will then be applied for signal detection. Biotin will be added followed by avidin with horseradish peroxidase (HRP). Finally lectin immunoreactivity will be detected using DAB as a chromagen. Capillary density analysis will be then assessed with the aid of an X25 objective and a 400 reticule grid attached to an eye piece. Each box in the 400 reticule grid measures 20μm x 20μm for a total area of 400μm^2). Four smCx areas per tissue section per animal will be analyzed. A photomontage is created using a SPOT digital camera connected to a Nikon E600 Eclipse microscope with a 20x objective. A computer-aided image analysis system (ImagePro Plus) is used to count marked antibody-positive capillaries between 4-25µm in diameter and determine the number per unit area of brain tissue.

BrdU labeling: BrdU will be administered in 60mg/kg doses IP once every other day from day 1-7 prior to TBI for a total of 4 injections. Following final injection, animals will be transcardially perfused and sections will be collected and processed for anti-BrdU (1:200) (see ICC and Double Immunofluorescence).

Isolation of CNS microvessels (MV): Male and female C57BL/6 mice (6-8 wks) will be purchased and acclimated overnight in the animal housing facility at the Elliman building to be examined for possible disease. Brain tissue will be removed within minutes of decapitation using sterile technique. Tissue will be homogenized in 10 volumes of MEM (Sigma, St. Louis, MO) and 10% FCS (Gibco BRL, Grand Island, NY) (pH 7.4) using a glass-Teflon homogenizer shaved to leave 0.25 µm between the plunger and the glass surface. We also have microhomogenizers that can be used to isolate MV from as little as 1-2 mouse brains. After 20 up-and-down strokes at 420 rpm, the homogenate will be centrifuged and the pellet suspended in MEM plus 15% Dextran 70 (Sigma). The suspension will be centrifuged at 5000 x g, 10 min. and the pellet suspended and filtered through a sterile 118 µm nylon mesh (Tetco, Braerclif Manor, NJ). The filtrate is passed through an 80 µm nylon mesh. MV are collected from the 40µm mesh (mice), washed vigorously from the mesh, and resuspended in DMEM plus 10% FCS. Yields equal about 0.2 mg MV protein/gram of starting material. MV have uniform diameter consistent with a predominant small capillaries and venules. Less than 2% are αSMA+ pial arterioles. MV are >95% viable by trypan blue exclusion. Purity is determined by visual examination and by measurement of γ-glutamyltranspepidase activity (Sigma, kit 545).

Polymerase Chain Reactions (PCR): Total RNA will be isolated using a single step guanidinium isothyocyanate procedure. Reverse transcription (RT) will be performed for 60 min. at 42°C followed by a 10

min. step at 80°C in a 30 µl reaction consisting of µg total RNA template, 2.5 µM oligonucleotide, 1 x PCR buffer, 3 mM MgCl and 0.5 mM each dNTP, 30U RNAse inhibitors, and 100 µ/ml murine leukemia virus (MULV) reverse transcriptase (Perkin-Elmer, Norwalk, CT). PCR is performed in 25 µl consisting of 2 µl of RT reaction (cDNA), 50 pmol each of primers (sense and antisense primers), 1X PCR buffer, 4 mM MgCl 2, 200 µM/liter each dNTP, and 2 U AmpliTaq polymerase (Perkin-Elmer). For exponential amplification, parameters for denaturation, annealing, and extension are: 5 min. at 95°C one cycle, then 30 cycles for 1 min. at 95°C, and 1 min. at 72°C, followed by a 72°C extension reaction for 10 min. Reaction will be performed in a Perkin-Elmer Thermal Cycler (GeneAmp PCR system 2400). β-actin internal control, co-amplified using sense (5" AACCCTAAGGCCAACCGTGAAA3') and antisense (5'TCATGAGGTAGTCTGTCAGGTC-3'). Controls include no RNA and no reverse transcriptase. For semi-quantification every primer pair will be tested at different cycle numbers to determine the linear range. Control mRNA levels will be measured at 23 cycles. Aliquots of 5 µl of biotinylated PCR product will be analyzed using a fluorescent digoxigenin detection ELISA kit (Boehringer Mannheim), according to manufacturer's protocol. All data will be normalized against control mRNA levels. PCR products are sub-cloned after gel purification then sequenced. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA housekeeping probe will be used for normalization of inducible genes. RNA blots: RNA (3 µg) will be applied to a 1.2% formaldehyde agarose gel, electrophoresed then transferred to Immobilon-N (Millipore). Blots will be sequentially probed with appropriated experimental and control cDNA labeled with 32pdCTP by random priming. Hybridizations will be conducted at 42C for 16hrs. in 50% formamide 0.75M NaCl, 75mM sodium citrate 0.1% SDS, 1mMEDTA, 10mM sodium phosphate pH6.8, 5x Denharts solution (Sigma), 10% dexham sulfate, and 100mg of salmon sperm DNA. Blots will be washed 3x at room temperature and 2x and visualized by autoradiography. **Quantitative real-time PCR:** Quantitative analysis of mRNA expression will be performed by RT- PCR using an ABI Prism 7700 sequence detection system (Perkin Elmer) and SYBR green master mix kit. Sequences of primers and probes will be as follows: VEGF reverse primer,5'-TGGAAGATGTCCACCAGGGT-3'; VEGFprobe, 5'/TET/AGCGCAGCTACTGCCA-TCCAATCG/TAM/-3; β-actin forward primer, 5-TCACCATGGATGATGATATCGC-3'; β-actin reverse primer, 5'-AAGCCGGCCTTGCACAT-3' and β-actin probe, 5'/FAM/CGCTCGTCCGTCGACA-ACGGCT/TAM/-3'. The primers for HIF-1α (TTTTACCATGCCCCAGATTCA and AGTGCTTCCATCGGAAGGACT), Actin (GCCCTGAGGCACTCTTCCA and ATGCCACAGGACTCCATGC).The data obtained are presented as the mean ratio of VEGF to β-actin mRNA (±SD) obtained from triplicate samples.

CBF Measurements: Prior to image acquisition, anesthesia will be induced by an intraperitoneal injection of a mixture of xylazine (12.5 mg/kg), ketamine (125 mg/kg) and atropine (0.08 mg/kg) to sedate the animals. The animal will be placed in a prone position on a cradle with a custom-built palate holder equipped with an adjustable nose cone and stereotaxic ear bars in order to minimize movement during MRI scans.

The animal's head will be positioned at the isocenter of a magnet. MRI scans were repeated at four time points. Baseline scans will be run before TBI is induced, and then at the 4th hour, 24th hour and 48th hour post-TBI. All MRI measurements will be performed on a 4.7-T horizontal-bore magnetic resonance spectrometer (Bruker AVANCE) with an 11.6-cm-bore actively shielded gradient coil set capable of producing a magnetic field gradient of up to 250 mT/m. A whole-body birdcage radiofrequency (RF) coil (inner diameter, 72 mm) will be used as the transmitter for homogeneous RF excitation, and a surface coil (30 mm diameter) will be used as the receiver, with active RF decoupling to avoid signal interference. Four sequences will be run in this set of experiments: T2-weighted imaging, T1-weighted imaging, and ASL for the measurement of flow and SWI to measure changes in oxygen saturation and flow, as well as for the determination of evidence of vascular damage and hemorrhage. All sequences will be run as in Shen et al (86) (attached).

In vitro hydroxylation assay. Cell lysates will be prepared from normal capillaries and capillaries isolated from injured animals as a source of HIF prolyl hydroxylases. Capillaries will be lysed in 1 ml of HEB buffer (20 mM Tris-HCl [pH 7.4], 5 mM KCl, 1.5 mM MgCl2) containing protease inhibitors and phosphatase inhibitors. For each 50-µl reaction, 200 µg of total protein will be used. A wheat germ in vitro transcription-translation (IVT)(Promega per manufacturer's directions) system will be used to generate unhydroxylated GHO and GHO(P3A) proteins to be used as substrate for in vitro hydroxylation activity. Each reaction will be carried out at 37°C for 15 min and terminated by adding 12.5 µl of 5X SDS loading buffer.

Generation of genetically manipulated mice: 1) neuron-specific knockdown of HIF-1α (75). Genetic ablation of HIF-1α at the systemic level results in embryonic lethality (E10.5) attributable to severe cardiovascular as well as other defects (99). A conditional knockdown approach was used to inactivate HIF-1α expression specifically in neurons. This deletion was achieved by crossing mice bearing loxP-containing

(floxed) HIF-1α alleles (HIF-1$\alpha^{F/F}$) with transgenic mice expressing Cre recombinase under control of the CaMKIIα promoter. Two independent lines of mice containing the Cre transgene were used, named R1ag#5 and L7ag#13 (100). Neuron-specific expression of Cre recombinase was demonstrated by crossing each line with ROSA26Sor–LacZ reporter mice. Immunostaining showed no differences between R1 and L7 in the pattern of ß-galactosidase expression at 3 weeks of age; both lines demonstrated specific expression of the reporter in most neurons. Southern blot analysis showed that the excision of floxed HIF-1α exon 2 was primarily restricted to forebrain areas without affecting the cerebellum. The majority of Cre(+):HIF-1$\alpha^{F/F}$ mice, denoted R1-HIF-1$\alpha^{\Delta/\Delta}$ or L7-HIF-1$\alpha^{\Delta/\Delta}$, were born without obvious abnormalities and survived into adulthood. Mice will be provided by Dr. Joseph LaManna.

Immunocytochemistry and beta-galactosidase staining. All histological and immunocytochemical analyses are conducted using 4 to 6 sections per animal with 3 to 6 areas of analysis per section, 4-6 animals (see methodology). Data is expressed as an average of each area of analysis. Between-group analyses are accomplished using one-way analysis of variance (ANOVA) with least significant difference post-hoc testing. Data are reported as mean ± SE. Significance is set at p-value < 0.05. Based on variability in these data from our previous studies (9) we can distinguish a difference in individual proteins and in capillary density with 95% power. Brains from *EMH-CreER^{T2}:ROSA26* mice are dissected from 2% paraformaldehyde-perfused mice and are wholemount stained or are vibratome sectioned (100 um) and stained free-floating overnight at 37°C with 1 mg/ml X-gal. Alternatively, we cut 10um cryostat sections and label the sections with anti-beta-galactosidase antibodies (Cappel) for co-localization with other markers.

Statistical Analysis:

Western Analysis. Protein is expressed as the average of samples blotted independently. Group analyses are accomplished using one-way analysis of variance (ANOVA) with least significant difference (LSD) post-hoc testing. Data is reported as mean ± SE. Significance is set at p-value < 0.05. We are able to detect significant changes in protein expression between groups (3 animals) with 95% power at 0.05.

CBF measurements. All data pertaining to CBF are expressed as the average of scans taken independently. CBF is expressed as mL/100g/min. Between group analyses are accomplished using one-way analysis of variance (ANOVA) with least significant difference (LSD) post-hoc testing. Data are reported as mean ± SE. Significance is set at p-value < 0.05. As previously reported (84), we were able to detect significant changes in CBF between groups using 6 animals per group with 95% power at $\alpha = 0.05$.

Behavioral Assessments. Behavioral data are expressed as the average latency of completing the task over three trials. Between group analyses are accomplished using one-way analysis of variance (ANOVA) with least significant difference (LSD) post-hoc testing. Data are reported as mean ± SE. Significance is set at p-value < 0.05. Due to the variability in behavior amongst individual animals, we have previously determined (12) that we can distinguish significant differences between TBI and control animals using 12 animals per group. Antagonist and agonist studies require 12-15 animals per group to show an improved performance with power of 90%. This allows us to distinguish a difference in latency of 2 min with 90% power at $\alpha = 0.05$. Additional mice may be required due to failure to exercise, death, or motoric disability following injury.

Vertebrate Animals

Description of involvement: We will use mice for the experiments. C57BL/6 mice will be tested to allow for comparison to the HIF-1 transgenic and knockdown mice that were derived on the same background and will be used in Aim #3. The experiments will include 4 mice per group (determined by power analysis) and are repeated 3 times. The animals are housed in virus free facilities under the supervision of the Wayne State University School of Medicine Animal Investigation Committee.

Justification for use: Animal models of traumatic brain injury (TBI) are useful experimental models that simulate TBI in humans. There are no mathematical models, computer simulations or in vitro systems that can substitute for the in vivo disease.

Many of the treatments available for TBI were first tested in preclinical studies using these models. The Marmarou model of closed head injury while somewhat variable allows for testing noninvasive treatment options such as the two protocols addressed in this proposal. Based on the work proposed, we expect to use approximately 20 mice per month.

Veterinary care: Animals are handled and cared for according to Federal, Institutional, and AAALAC guidelines. Dr. Elizabeth Dawe, DVM, Professor of Pathology, supervises the animal care facility in the Elliman Clinical Research Building, and her staff consists of more than 20 qualified veterinary technicians who are available 24/7.

Procedures to minimize discomfort and pain: All procedures are performed under anesthesia. Animals will be anesthetized with 5% halothane in 2% oxygen prior to intubation, and then maintained on 1.5% halothane via a mask and spontaneous breathing during the procedure. Adequate levels of anesthesia will be assured by using corneal reflex, and respiration pattern. Animals will be sacrificed by decapitation. This technique is used to avoid the effect of anesthesia on CNS capillaries.

Techniques: For head injury, animals are anesthetized as detailed above and a mid-sagittal scalp incision will be performed. The underlying muscles will be retracted laterally. Cranioplastic cement will be used to attach a 10mm diameter X 3 mm thick, round metal helmet directly to the skull over the sagittal suture and between the coronal and lambdoidal sutures. The helmet is used to distribute the applied force over the surface of the parietal bones, thus preventing skull fractures with penetrating brain injury. After the cement is allowed to dry for 3 min, the animals will be placed prone onto a platform as described in the Acceleration Impact Trauma Model of Marmarou (78). After 30-40 seconds of placement, 250g of weight (mice) contained in a hollow plastic cylinder will be dropped directly onto the helmet from a height of 2 cm (mice). Following a brief convulsion and respiratory arrest, most animals restart breathing on their own. However, in some cases, the use of a rodent respirator or CPR is necessary prior to spontaneous breathing. Using these precautions, mortality has been reduced to less than 5%. In some animals after impact, the helmet will be removed and the skin sutured only if the skull shows no evidence of fractures. After suturing the skin, sensory cutaneous and evoked motor responses will be tested. Usually the intubation tube is removed at 10 min post trauma and only animals that are able to right themselves before 30 min after injury will be included in the study. Brain and leg muscle temperatures will be taken routinely, in some instances up to 24 hrs post injury. Brain temperature fluctuates only 1.5°C, and muscle temperature 1.3°C, during this time period. The extent of posttraumatic neurological impairment was assessed at defined time intervals after trauma (t = 1 h, 4 h, 24 h, and 7 days) using a standardized *Neurological Severity Score* (NSS).

Method of euthanasia: Euthanasia is performed by CO_2 inhalation administered according to institutional guidelines. Animals exhibiting excessive neurological damage or signs of infection will be removed from the study and euthanized.

Bibliography

1. Veterans Health Initiative Independent Study Course Traumatic Brain Injury, January, 2004. pp. 1-18.
2. Wallsten S, Kosec K. 2005. The Economic Costs of the War in Iraq. AEI-Brookings Joint Center Working Paper No. 05-19.
3. Traumatic Brain Injury. Last modified Sep 21, 2007. Last accessed Jun 01, 2009. www.cdc.gov/ncipc/factsheets/tbi.htm.
4. Scheid R, Walther K, Guthke T, Preul C, von Cramon DY. 2006. Cognitive sequelae of diffuse axonal injury. Arch Neurol 63:418-424.
5. Soeda A, Nakashima T, Okumura A, Kuwata K, Shinoda J, Iwama T. 2005. Cognitive impairment after traumatic brain injury: a functional magnetic resonance imaging study using the Stroop task. Neuroradiology. 47:501-6.
6. Marmarou A. 2007. A review of progress in understanding the pathophysiology and treatment of brain edema. Neurosurg Focus 22(5):E1.
7. Büki A, Povlishock JT. 2006. All roads lead to disconnection?--Traumatic axonal injury revisited. Acta Neurochir (Wien) 148(2): 181-93; discussion 193-4.
8. Zitnay GA, Zitnay KM, Povlishock JT, Hall ED, Marion DW, Trudel T, Zafonte RD, Zasler N, Nidiffer FD, DaVanzo J, Barth JT. 2008. Traumatic brain injury research priorities: the Conemaugh International Brain Injury Symposium. J Neurotrauma 25(10): 1135-52.
9. Ward NL. Lamanna JC. 2004. The neurovascular unit and its growth factors: coordinated response in the vascular and nervous system. Neurol Res 26:870-883.
10. LaManna JC, Chavez JC, Pichiule P. 2004. Structural and functional adaptation to hypoxia in the rat brain. J Exp Biol 2007:163-169.
11. Dore-Duffy P, LaManna JC. 2007. Physiologic angiodynamics in the brain. Antioxid Redox Signal 9:1363-1371.
12. Ding YH, Li J, Zhou Y, Rafols JA, Clark JC, Ding Y. 2006. Cerebral angiogenesis and expression of angiogenic factors in aging rats after exercise. Curr Neurovasc Res. 3:15-23.
13. Ding YH, Luan XD, Li J, Rafols JA, Guthinkonda M, Diaz FG, Ding Y. 2004. Exercise-induced overexpression of angiogenic factors and reduction of ischemia/reperfusion injury in stroke. Curr Neurovasc Res 1:411-420.
14. Kreipke CW, Morgan R, Kallakuri S, Rafols JA. 2007. Behavioral pre-conditioning enhances angiogenesis and cognitive outcome after brain trauma. Neurol Res 29:388-394.
15. Pickard JD, Hutchinson PJ, Coles JP, Steiner LA, Johnston AJ, Fryer TD, Coleman MR, Smielewski P, Chatfield DA, Aigbirhio F, Williams GB, Rice K, Clark JC, Salmond CH, Sahakian BJ, Bradley PG, Carpenter TA, Salvador R, Pena A, Gillard JH, Cunningham AS, Piechnik S, Czosnyka M, Menon DK. 2005. Imaging of cerebral blood flow and metabolism in brain injury in the ICU. Acta Neurochir Suppl 95:459-464.
16. Bonne O, Gilboa A, Louzoun Y, Kempf-Sherf O, Katz M, Fishman Y, Ben-Nahum Z, Krausz Y, Bocher M, Lester H, Chisin R, Lerer B. 2003. Cerebral blood flow in chronic symptomatic mild traumatic brain injury. Psychiatry Res 124:141-152.
17. Petrov T, Rafols JA. 2001. Acute alterations of endothelin-1 and iNOS expression and control of the brain microcirculation after head trauma. Neurol Res 23:139-143.

18. Dore-Duffy P, Wang X, Mehedi A, Kreipke CW, Rafols JA. 2007. Differential expression of capillary VEGF isoforms following traumatic brain injury. Neurol Res 29:395-403.

19. Ding YH, Li J, Rafols JA, Ding Y. 2004. Reduced brain edema and matrix metalloproteinase (MMP) expression by pre-reperfusion infusion into ischemic territory in rat. Neurosci Lett 372(1-2):35-9.

20. Papavassiliou E, Gogate N, Pooescholdt M, Heiss JD, Walbridge S, Edwards NA, Oldfield EH, Merrill MJ. 1997. Vascular endothelial growth factor (vascular permeability factor) expression in injured rat brain. J Neuroscience Res. 49: 451-60

21. Skold MK, von Gertten C, Sandberg-Nordqvist AC, Mathiesen T, Holmin S. 2005. VEGF and VEGF receptor expression after experimental brain contusion in rat. J Neurotrauma 22: 353-67.

22. Skold MK, Risling M, Holmin S. 2006. Inhibition of vascular endothelial growth factor receptor 2 activity in experimental brain contusions aggravates injury outcome and leads to early increased neuronal and glial degenerations. Eur J Neurosci 23: 21-34.

23. Dore-Duffy P, Owen C, Balabanov R, Murphy S, Beaumont T, Rafols JA. 2000. Pericyte migration from the vascular wall in response to traumatic brain injury. Microvasc Res 60:55-69.

24. Balabanov R, Goldman H, Murphy S, Pellizon G, Owen C, Rafols J, Dore-Duffy P. 2001. Endothelial cell activation following moderate traumatic brain injury. Neurol Res 23:175-82.

25. Dore-Duffy P, Balabanov R, Beaumont T, Hritz MA, Harik SI, LaManna JC. 1999. Endothelial activation following prolonged hypobaric hypoxia. Microvasc Res 57:75-85

26. Bartnik BL, Lee SM, Hovda DA, Sutton RL. 2007. The fate of glucose during the period of decreased metabolism after fluid percussion injury: a 13C NMR study. J Neurotrauma 24:1079-92.

27. Bartnik BL, Hovda DA, Lee PW. 2007. Glucose metabolism after traumatic brain injury: estimation of pyruvate carboxylase and pyruvate dehydrogenase flux by mass isotopomer analysis. J Neurotrauma 24(1):181-94.

28. Goodman JC, Valadka AB, Gopinath SP, Uzura M, Robertson CS. 1999. Extracellular lactate and glucose alterations in the brain after head injury measured by microdialysis. Crit Care Med 27(9):1965-73.

29. Anderson J, Sandhir R, Hamilton ES, Berman NE. 2009. Impaired Expression of Neuroprotective Molecules in the HIF-1-alpha Pathway following Traumatic Brain Injury in Aged Mice. J Neurotrauma [Epub ahead of print].

30. Park E, Bell JD, Siddiq IP, Baker AJ. 2009. An analysis of regional microvascular loss and recovery following two grades of fluid percussion trauma: a role for hypoxia-inducible factors in traumatic brain injury. J Cereb Blood Flow Metab 29(3):575-84.

31. Shein NA, Grigoriadis N, Alexandrovich AG, Simeonidou C, Spandou E, Tsenter J, Yatsiv I, Horowitz M, Shohami E. 2008. Differential neuroprotective properties of endogenous and exogenous erythropoietin in a mouse model of traumatic brain injury. J Neurotrauma 25(2):112-23.

32. Wakui, S., M. Furusato, H. Ohshige, and S. Ushigome. 1993. Endothelial-pericyte interdigitations in rat subcutaneous disc implanted angiogenesis. Microvasc Res 46:19-27.

33. Hirschi, K.K. and P.A. D'Amore. 1997. Control of angiogenesis by the pericyte: molecular mechanisms and significance. Exs 79:419-28

34. Black JE, Isaacs KR, Anderson BJ, Alcantara AA, Greenough WT. 1990. Learning causes synaptogenesis, whereas motor activity causes angiogenesis, in cerebellar cortex of adult rats. Proc Natl Acad Sci U S A. 87:5568-72.

35. Isaacs KR, Anderson BJ, Alcantara AA, Black JE, Greenough WT. 1992. Exercise and the brain:

angiogenesis in the adult rat cerebellum after vigorous physical activity and motor skill learning. J Cereb Blood Flow Metab. 12:110-9.

36. van Praag H, Lucero MJ, Yeo GW, Stecker K, Heivand N, Zhao C, Yip E, Afanador M, Schroeter H, Hammerstone J, Gage FH. 2007. Plant-derived flavanol (-)epicatechin enhances angiogenesis and retention of spatial memory in mice. J Neurosci 27:5869-78.
37. Lu D, Qu C, Goussev A, Jiang H, Lu C, Schallert T, Mahmood A, Chen J, Li Y, Chopp M. 2007. Statins increase neurogenesis in the dentate gyrus, reduce delayed neuronal death in the hippocampal CA3 region, and improve spatial learning in rat after traumatic brain injury. J Neurotrauma 24:1132-1146.
38. LaManna JC, Harik SL. 1997. Brain metabolic and vascular adaptations to hypoxia in the rat. Review and update. Adv Exp Med Biol. 428:163-167
39. Papandreou I, Powell A, Lim AL, Denko N. 2005. Cellular reaction to hypoxia: sensing and responding to an adverse environment. Mutat Res 569:87-100.
40. Wencel, M., Katyshev, V., Cleary, K., Mehedi, A., Dore-Duffy, P. 2009. Adaptive angiogenesis in response to normobaric hypoxia is protective in EAE. J. Neurochem 108 (Suppl 1): 153.
41. Glenn TC, Kelly DF, Boscardin WJ, McArthur DL, Vespa P, Oertel M, Hovda DA, Bergsneider M, Hillered L, Martin NA. 2003. Energy dysfunction as a predictor of outcome after moderate or severe head injury: indices of oxygen, glucose, and lactate metabolism. J Cereb Blood Flow Metab 23:1239-1250.
42. Moppett IK, Hardman JG. 2007. Modeling the causes of variation in brain tissue oxygenation. Anesth Analg 105:1104-1112.
43. McHugh GS, Engel DC, Butcher I, Steyerberg EW, Lu J, Mushkudiani N, Hernandez AV, Marmarou A, Maas AI, Murray GD. 2007. Prognostic value of secondary insults in traumatic brain injury: results from the IMPACT study. J Neurotrauma 24:287-293.
44. Mazzeo AT, Bullock R. 2007. Monitoring brain tissue oxymetry: Will it change management of critically ill neurologic patients? J Neurol Sci 261:1-9.
45. Hlatky R, Valadka AB, Goodman JC, Contant CF, Robertson CS. 2004. Patterns of energy substrates during ischemia measured in the brain by microdialysis. J Neurotrauma 21(7):894-906.
46. Stiefel MF, Udoetuk JD, Storm PB, Sutton LN, Kim H, Dominguez TE, Helfaer MA, Huh JW. 2006. Brain tissue oxygen monitoring in pediatric patients with severe traumatic brain injury. J Neurosurg 105:281-286.
47. Bardt TF, Unterberg AW, Hartl R, Kiening KL, Schneider GH, Lanksch WR. 1998. Monitoring of brain tissue PO2 in traumatic brain injury: effect of cerebral hypoxia on outcome. Acta Neurochir Suppl 71:153-156.
48. Alves OL, Bullock R, Clausen T, Reinert M, Reeves TM. 2005. Concurrent monitoring of cerebral electrophysiology and metabolism after traumatic brain injury: an experimental and clinical study. J Neurotrauma 22:733-749.
49. Shahlaie K, Boggan JE, Latchaw RE, Ji C, Muizelaar JP. 2009. Posttraumatic vasospasm detected by continuous brain tissue oxygen monitoring: treatment with intraarterial verapamil and balloon angioplasty. Neurocrit Care 10(1):61-9.
50. Bouma GJ, Muizelaar JP, Bandoh K, Marmarou A. 1992. Blood pressure and intracranial pressure-volume dynamics in severe head injury: relationship with cerebral blood flow. J Neurosurg 77:15-19.

51. Chi JH, Knudson MM, Vassar MJ, McCarthy MC, Shapiro MB, Mallet S, Holcroft JJ, Moncrief H, Noble J, Wisner D, Kaups KL, Bennick LD, Manley GT. 2006. Prehospital hypoxia affects outcome in patients with traumatic brain injury: a prospective multicenter study. J Trauma 61:1134-1141.

52. Schmoker JD, Zhuang J, Shackford SR. 1992. Hemorrhagic hypotension after brain injury causes an early and sustained reduction in cerebral oxygen delivery despite normalization of systemic oxygen delivery. J Trauma 32:714-720.

53. Bramlett HM, Green EJ, Dietrich WD. 1999. Exacerbation of cortical and hippocampal CA1 damage due to posttraumatic hypoxia following moderate fluid-percussion brain injury in rats. J Neurosurg. 91:653-659.

54. Bramlett HM, Dietrich WD, Green EJ. 1999. Secondary hypoxia following moderate fluid percussion brain injury in rats exacerbates sensorimotor and cognitive deficits. J Neurotrauma. 16:1035-1047.

55. Van Putten HP, Bouwhuis MG, Muizelaar JP, Lyeth BG, Berman RF. 2005. Diffusion-weighted imaging of edema following traumatic brain injury in rats: effects of secondary hypoxia. J Neurotrauma 22:857-872.

56. Mikrogianakis A, Shaye RE, Griffin P, Kawesa S, Lockwood J, Gendron NH, Gaboury I, Merali Z, Mackenzie AE, Hutchison JS. 2007. Hypoxia alters the expression of inhibitor of apoptosis proteins after brain trauma in the mouse. J Neurotrauma 24:338-353.

57. Truettner JS, Hu B, Alonso OF, Bramlett HM, Kokame K, Dietrich WD. 2007. Subcellular stress response after traumatic brain injury. J Neurotrauma 24:599-612.

58. O'Reilly JP, Jiang C, Haddad GG. 1995. Major differences in response to graded hypoxia between hypoglossal and neocortical neurons. Brain Res 683:179-186.

59. Milton SL, Nayak G, Lutz PL, Prentice HM. 2006. Gene transcription of neuroglobin is upregulated by hypoxia and anoxia in the brain of the anoxia-tolerant turtle Trachemys scripta. J Biomed Sci 13:509-514.

60. Ameri K, Lewis CE, Raida M, Sowter H, Hai T, Harris AL. 2005. Anoxic induction of ATF-4 through HIF-1-independent pathways of protein stabilization in human cancer cells. Blood 103:1876-1882.

61. Salim A, Nacamuli RP, Morgan EF, Giaccia AJ, Longaker MT. 2004. Transient changes in oxygen tension inhibit osteogenic differentiation and Runx2 expression in osteoblasts. J Biol Chem 279:40007-400016.

62. Jiang C, Xia Y, Haddad GG. 1992. Role of ATP-sensitive K+ channels during anoxia: major differences between rat (newborn and adult) and turtle neurons. J Physiol 448:599-612.

63. Whitbeck C, Barreto M, Horan P, Levin SS, Levin RM. 1999. Rabbit versus rat urinary bladder: effects of in vitro hypoxia. Pharmacology 59:156-164.

64. Seifalian AM, El-Desoky H, Delpy DT, Davidson BR. 2001. Effect of graded hypoxia on the rat hepatic tissue oxygenation and energy metabolism monitored by near-infrared and 31P nuclear magnetic resonance spectroscopy. FASEB J 15:2642-2648.

65. Archer SL, Freude KA, Shultz PJ. 1995. Effect of graded hypoxia on the induction and function of inducible nitric oxide synthase in rat mesangial cells. Circ Res 77:21-28.

66. Swain DP. 2000. Energy cost calculations for exercise prescription: an update. Sports Med 30:17-22.

67. Schiff SJ, Somjen GG. 1987. The effect of graded hypoxia on the hippocampal slice: an in vitro model of the ischemic penumbra. Stroke 18:30-37.

68. Sharp FR, Ran R, Lu A, Tang Y, Strauss KI, Glass T, Ardizzone T, Bernaudin M. 2004. Hypoxic preconditioning protects against ischemic brain injury. NeuroRx. 1:26-35.

69. Lu GW, Yu S, Li RH, Cui XY, Gao CY. 2005. Hypoxic preconditioning: a novel intrinsic cytoprotective strategy. Mol Neurobiol 31:255-271.

70. Grimm C, Wenzel A, Acar N, Keller S, Seeliger M, Gassmann M. 2006. Hypoxic preconditioning and erythropoietin protect retinal neurons from degeneration. Adv Exp Med Biol 588:119-131.

71. Chen L, Miller FL, Williams JJ, Alexander CM, Domino KB, Marshall C, Marshall BE. 1985. Hypoxic pulmonary vasoconstriction is not potentiated by repeated intermittent hypoxia in closed chest dogs. Anesthesiology. 63:608-610.

72. Park AM, Nagase H, Kumar SV, Suzuki YJ. 2007. Effects of intermittent hypoxia on the heart. Antioxid Redox Signal 9:723-729.

73. Onyszchuk G, He YY, Berman NE, Brooks WM. 2008. Detrimental effects of aging on outcome from traumatic brain injury: a behavioral, magnetic resonance imaging, and histological study in mice. J Neurotrauma 25(2): 153-71.

74. Belaidi E, Beguin PC, Ribuot C, Godin-Ribuot D. 2006. Hypoxic preconditioning: role of transcription factor HIF-1alpha Ann Cardiol Angeiol (Paris) 55:70-73.

75. Xu K, LaManna JC. 2006. Chronic hypoxia and the cerebral circulation. J Appl Physiol 100:725-730.

76. Verweij BH, Amelink GJ, Muizelaar JP. 2007. Current concepts of cerebral oxygen transport and energy metabolism after severe traumatic brain injury. Prog Brain Res. 161:111-124.

77. Buchele G, De Backer D, Vincent JL. 2007. The microcirculation in critically-ill patients. Acta Clin Belg 62:15-20.

78. Marmarou A, Foda MA, van den Brink W, Campbell J, Kita H, Demetriadou K. 1994. A new model of diffuse brain injury in rats. Part I: Pathophysiology and biomechanics. J Neurosurg 80:291-300.

79. Rafols JA, Morgan R, Kallakuri S, Kreipke CW. 2007. Extent of nerve cell injury in Marmarou's model compared to other brain trauma models. Neurol Res 29(4): 348-55.

80. Kreipke CW, Rafols JA, et al. Extent of hypoperfusion in murine model of traumatic brain injury. Neurotrauma. [In press].

81. Ding Y, Li J, Luan X, Ding YH, Lai Q, Rafols JA, Phillis JW, Clark JC, Diaz FG. 2004. Exercise pre-conditioning reduces brain damage in ischemic rats that may be associated with regional angiogenesis and cellular overexpression of neurotrophin. Neuroscience 124: 583-591.

82. Hayashi T, Abe K, Itoyama Y. 1998. Reduction of ischemic damage by application of vascular endothelial growth factor in rat brain after transient ischemia. J Cereb Blood Flow Metab. 18(8): 887-95.

83. Gu W, Brannstrom T, Jiang W, Bergh A, Wester P. 2001. Vascular endothelial growth factor-A and –C protein up-regulation and early angiogenesis in a rat photothrombotic ring stroke model with spontaneous reperfusion. Acta Neuropathol. 102:216-26.

84. Rafols JA, Morgan R, Kallakuri S, Kreipke CW. 2007. Extent of nerve cell injury in Marmarou's model compared to other brain trauma models. Neurol Res 29:348-355.

85. Morgan R, Kreipke CW, Roberts G, Bagchi M, Rafols JA. 2007. Neovascularization following traumatic brain injury: possible evidence for both angiogenesis and vasculogenesis. Neurol Res 29:375-381.

86. Shen Y, Kou Z, Kreipke CW, Petrov T, Hu J, Haacke EM. 2007. In vivo measurement of tissue damage, oxygen saturation changes and blood flow changes after experimental traumatic brain injury in rats using Susceptibility Weighted Imaging (SWI). Magn Reson Imaging 25:219-227.

87. Bell EL, Chandel NS. 2007. Mitochondrial oxygen sensing: regulation of hypoxia-inducible factor by mitochondrial generated reactive oxygen species. Essays Biochem 43:17-27.

88. Hagen T, Taylor CT, Lam F, Moncada S. 2003. Redistribution of intracellular oxygen in hypoxia by nitric oxide: effect on HIF1alpha. Science 302:1975-1978.

89. Singh IN, Sullivan PG, Deng Y, Mbye LH, Hall ED. 2006. Time course of post-traumatic mitochondrial oxidative damage and dysfunction in a mouse model of focal traumatic brain injury: implications for neuroprotective therapy. J Cereb Blood Flow Metab 26(11): 1407-18.

90. Gurkoff GG, Giza CC, Hovda DA. 2006. Lateral fluid percussion injury in the developing rat causes an acute, mild behavioral dysfunction in the absence of significant cell death. Brain Res 1077: 24-36.

91. Witgen BM, Lifshitz J, Smith ML, Schwarzbach E, Liang SL, Grady MS, Cohen AS. 2005. Regional hippocampal alteration associated with cognitive deficit following experimental brain injury: a systems, network and cellular evaluation. Neuroscience 133: 1-15.

92. Hong J, Cui J, Zhou Y, Gao J. 2002. Study on cognition disorder and morphologic change of neurons in hippocampus area following traumatic brain injury in rats. Chin J Traumatol 5: 36-39.

93. Milner R, Hung S, Erokwu B, Dore-Duffy P, LaManna JC, del Zoppo GJ. 2008. Increased expression of fibronectin and the alpha 5 beta 1 integrin in angiogenic cerebral blood vessels of mice subject to hypobaric hypoxia. Mol Cell Neurosci 38(1):43-52.

94. Scopel D, Fochesatto C, Cimarosti H, Rabbo M, Bello-Klein A, Salbego C, Netto CA, Siqueira IR. 2006. Exercise intensity influences cell injury in rat hippocampal slices exposed to oxygen and glucose deprivation. Brain Res Bull 71:155-159.

95. Kleim JA, Jones TA, Schallert T. 2003. Motor enrichment and the induction of plasticity before or after brain injury. Neurochem Res 28:1757-1769.

96. Auriat AM, Grams JD, Yan RH, Colbourne F. 2004. Forced exercise does not improve recovery after hemorrhagic stroke in rats. Brain Res 1109:183-191.

97. Leifflen D, Poquin D, Savourey G, Barraud PA, Raphel C, Bittel J. 1997. Cognitive performance during short acclimation to severe hypoxia. Aviat Space Environ Med. 68:993-7.

98. Lopez-Ramos JC, Yi PJ, Eleore L, Madronal N, Rueda A, Delgado-Garcia JM. J Appl Physiol 103:1479-87.

99. Zhang JX, Lu XJ, Wang XC, Li W, Du JZ. 2006. Intermittent hypoxia impairs performance of adult mice in the the two-way shuttle box but not in the Morris water maze. J Neurosci Res. 84:228-35.

100. Sen Gupta J, Matthew I, Gopinath PM. 1979. Effect of physical training at moderate altitude (1850m) on hypoxic tolerance. Aviat Space Environ Med. 50:714-716.

101. Maeda M, Hasebe Y, Egawa K, Shibanuma M, Nose K. 2006. Inhibition of angiogenesis and HIF-1alpha activity by antimycin A1. Biol Pharm Bull 29:1344-1348.

102. Pili R, Kruszewski MP, Hager BW, Lantz J, Carducci MA. 2001. Combination of phenylbutyrate and 13-cis retinoic acid inhibits prostate tumor growth and angiogenesis. Cancer Res 61:1477-85.

103. Kato Y, Yoshimura K, Shin T, Verheul H, Hammers H, Sanni TB, Salumbides BC, Van Erp K, Schulick R, Pili R. 2007. Synergistic in vivo antitumor effect of the histone deacetylase inhibitor MS-275 in combination with interleukin 2 in a murine model of renal cell carcinoma. Clin Cancer Res 13:4538-46.

104. Kim SH, Kim KW, Jeong JW. 2007. Inhibition of hypoxia-induced angiogenesis by sodium butyrate, a histone deacetylase inhibitor, through hypoxia-inducible factor-1alpha suppression. Oncol Rep 17:793-7.

105. Warnecke C, Griethe W, Weidemann A, Jurgensen JS, Willam C, Bachmann S, Ivashchenko Y, Wagner I, Frei U, Wiesener M, Eckardt KU. 2003. Activation of the hypoxia-inducible factor-pathway and stimulation of angiogenesis by application of prolyl hydroxylase inhibitors. FASEB J 17:1186–1188.

106. Demougeot C, Van Hoecke M, Bertrand N, Prigent-Tessier A, Mossiat C, Beley A, Marie C. 2004. Cytoprotective efficacy and mechanisms of the liposoluble iron chelator 2,2'-dipyridyl in the rat photothrombotic ischemic stroke model. J Pharmacol Exp Ther 311:1080–1087.

107. Baranova O, Miranda LF, Pichiule P, Dragatsis I, Johnson RS, Chavez JC. 2007. Neuron-specific inactivation of the hypoxia inducible factor 1 alpha increases brain injury in a mouse model of transient focal cerebral ischemia. J Neurosci 27:6320-32.

108. Van Hoecke M, Prigent-Tessier A, Bertrand N, Prevotat L, Marie C, Beley A. 2005. Apoptotic cell death progression after photothrombotic focal cerebral ischaemia: effects of the lipophilic iron chelator 2,2'-dipyridyl. Eur J Neurosci 22:1045–1056.

109. Siddiq A, Ayoub IA, Chavez JC, Aminova L, Shah S, LaManna JC, Patton SM, Connor JR, Cherny RA, Volitakis I, Bush AI, Langsetmo I, Seeley T, Gunzler V, Ratan RR. 2005. Hypoxia-inducible factor prolyl 4-hydroxylase inhibition. A target for neuroprotection in the central nervous system. J Biol Chem 280:41732–41743.

110. Freret T, Valable S, Chazalviel L, Saulnier R, MacKenzie ET, Petit E, Bernaudin M, Boulouard M, Schumann-Bard P. 2006. Delayed administration of deferoxamine reduces brain damage and promotes functional recovery after transient focal cerebral ischemia in the rat. Eur J Neurosci 23:1757–1765.

111. Majamaa K, Turpeenniemi-Hujanen TM, Latipaa P, Gunzler V, Hanauske-Abel HM, Hassinen IE, Kivirikko KI. 1985. Differences between collagen hydroxylases and 2-oxoglutarate dehydrogenase in their inhibition by structural analogues of 2-oxoglutarate. Biochem J 229:127-133.

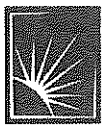
112. Mogi M, Li JM, Iwanami J, Min LJ, Tsukuda K, Iwai M, Horiuchi M. 2006. Angiotensin II type-2 receptor stimulation prevents neural damage by transcriptional activation of methyl methanesulfonate sensitive 2. Hypertension 48:141-148.

113. Koshiji M, Huang LE. 2004. Dynamic balancing of the dual nature of HIF-1alpha for cell survival. Cell Cycle 3:853-4.

114. Pan Y, Mansfield KD, Bertozzi CC, Rudenko V, Chan DA, Giaccia AJ, Simon MC. 2007. Multiple factors affecting cellular redox status and energy metabolism modulate hypoxia-inducible factor prolyl hydroxylase activity in vivo and in vitro. Mol Cell Biol 27:912-25.

115. Chen C, Hu Q, Yan J, Yang X, Shi X, Lei J, Chen L, Huang H, Han J, Zhang JH, Zhou C. 2009. Early inhibition of HIF-1alpha with small interfering RNA reduces ischemic-reperfused brain injury in rats. Neurobiol Dis 33(3): 509-17.

116. Dragatsis I, Zeitlin S. 2000. CaMKIIalpha-Cre transgene expression and recombination patterns in the mouse brain. Genesis. 26:133-5.



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May 20, 2009

Paula Dore-Duffy, PhD
Wayne State University
School of Medicine
3124 Elliman Bldg.
421 E Canfield
Detroit, MI 48201

Dear Paula:

I am pleased to write this letter in support of your studies on adaptive angiogenesis, and hypoxia following TBI. This collaboration is a natural extension from our longstanding and ongoing collaboration on physiological angiodynamics in an in vivo model of hypoxia. I have reviewed your preliminary data and concur with your hypothesis that restoration of proper physiological angiogenesis will enhance neuroprotection, thus mitigating the extent of secondary injury and sparing cognitive deficit following TBI, and that therapeutic and behavioral intervention that promotes physiological adaptive angiogenesis will have a profound effect on clinical outcome and long term recovery following TBI.

I look forward to our continued collaboration and to the studies proposed in this application.

Yours truly,

Joe

Joseph C. LaManna, PhD
Professor of Physiology & Biophysics,
Neurology and Neurosciences

JCL:sf

PHS 398 Checklist

OMB Number: 0925-0001
Expiration Date: 9/30/2007

1. Application Type:

From SF 424 (R&R) Cover Page. The responses provided on the R&R cover page are repeated here for your reference, as you answer the questions that are specific to the PHS398.

* Type of Application:

☒ New ☐ Resubmission ☐ Renewal ☐ Continuation ☐ Revision

Federal Identifier:

2. Change of Investigator / Change of Institution Questions

☐ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:
* First Name:
Middle Name:
* Last Name:
Suffix:

☐ Change of Grantee Institution

* Name of former institution:

3. Inventions and Patents (For renewal applications only)

* Inventions and Patents: Yes ☐ No ☐

If the answer is "Yes" then please answer the following:

* Previously Reported: Yes ☐ No ☐

OMB Number. 0925-0001
Expiration Date: 9/30/2007

4. * Program Income

Is program income anticipated during the periods for which the grant support is requested?

☐ Yes ☒ No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

5. Assurances/Certifications (see instructions)

In agreeing to the assurances/certification section 18 on the SF424 (R&R) form, the authorized organizational representative agrees to comply with the policies, assurances and/or certifications listed in the agency's application guide, when applicable. Descriptions of individual assurances/certifications are provided at: http://grants.nih.gov/grants/funding/424

If unable to certify compliance , where applicable, provide an explanation and attach below.

Explanation: Add Attachment | Delete Attachment | View Attachment